THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXV JULY, 1949 NUMBER 4

THE REACTION PRODUCED IN THE PULMONARY ARTERIES BY EMBOLI OF COTTON FIBERS*

WILLIAM C. VON GLAHN, M.D., and JOHN W. HALL, M.D.

(From the Department of Pathology of New York University College of Medicine, and Bellevue Hospital, New York, N.Y.)

In the routine examination of sections of lungs from necropsies over a period of 20 years, we have observed, as an incidental finding in 6 cases, foreign bodies in the smaller branches of the pulmonary arteries. These foreign particles were slender and had a faintly greenish tinge; they did not stain with the dyes used in routine histologic preparations and were doubly refractive. They did not cause thrombus formation. The foreign bodies usually lay against the intimal surface of the artery and in some instances a few large mononuclear cells and several multinucleated giant cells of the foreign body type had collected about them (Fig. 1).

When the histories of these patients were studied, there was one feature common to all: Each had received either one or more intravenous injections of physiologic saline or glucose solution or blood transfusions, within a period of not more than 10 days prior to death. The foreign particles were considered to be fragments of cotton that were contained in the solution injected and that had come either from the gauze through which the solution had been filtered, or from particles of a cotton stopper that adhered to the mouth of the flask.

To test the correctness of the impression that these foreign particles were cotton fibers and to study in detail the lesions that might be produced by them in the pulmonary arteries, the following experiments were performed.

PROCEDURE AND METHODS

A small mass of cotton, either absorbent or nonabsorbent, was immersed in physiologic saline solution and cut into very minute fragments. These were of such a size as to be readily drawn into a syringe through a no. 19 gauge hypodermic needle. Each cubic centimeter of saline solution contained approximately 0.02 cc. of cotton fibers. The physio-

* Received for publication, July 6, 1948.

logic saline solution containing the cotton fibers was then sterilized in the autoclave at a pressure of 15 lbs. for 30 minutes.

Sixty-two adult albino rats were used in the experiment. Under ether anesthesia, an iliac vein was exposed, and approximately 1 cc. of the saline solution with the suspended cotton fibers was injected. When the injection did not proceed rapidly, it was found that the fibers had settled and jammed the plunger of the syringe. When this occurred another injection was made into the other iliac vein. There were no untoward effects from the injections and each animal appeared normal as soon as it recovered from the anesthesia. Rats in groups of 3 to 5 were sacrificed at varying intervals, some at the end of 24 and 48 hours and others at approximately weekly intervals thereafter for the first 2 months and at longer intervals between the third and sixth months.

At necropsy, 3 or 4 specimens of tissue were obtained from the lungs of each animal; these were fixed in Zenker's solution without the addition of acetic acid or formalin, and in 10 per cent formalin solution. Three to 5 sections were cut from each paraffin-impregnated block of tissue. In some instances, the block was sectioned serially. The routine stains were hematoxylin and eosin, and Weigert's stain for elastic tissue counterstained with hematoxylin and eosin. Occasionally, Wilder's silver stain counterstained with azocarmine-aniline blue was used.

Results

Some of the animals were injected with absorbent cotton, others with nonabsorbent cotton. The changes observed were the same, and a single description of the results will suffice for both groups. Before discussing the results it is necessary to review briefly the structure of the pulmonary artery of the rat.

The first branches of the pulmonary artery to enter the various lobes of the lung have walls of uniform thickness, the media being well developed. In the branches of medium size, the wall of the artery often undergoes an abrupt change in thickness, owing to a sudden thinning of the media. This change may involve both sides of the vessel as seen in longitudinal or tangential section, although in many the thinning of the media is restricted to a segment of the wall. From these vessels of medium size, branches are at times given off abruptly at right angles. The arterioles have walls of uniform width. The precapillary branches consist of endothelium, a single layer of elastic tissue, and adventitia.

The injected cotton fibers were lodged chiefly in the arterioles and in the precapillary branches. At the end of 24 hours many of the cotton

fibers were found in apposition to the endothelium and without any reaction about them. In other instances, large mononuclear cells were densely packed about the cotton fibers, and about some of these vessels were collections of similar cells (Fig. 2). These large mononuclear cells completely filled the lumina of the precapillary branches. The elastica was stretched and often ruptured at one point, permitting the mononuclear cells within the lumen to stream through and merge with any cells that had collected about the vessel. No multinucleated cells were present nor was there any thrombus formation. Forty-eight hours after the injection, most of the cotton fibers were surrounded by large mononuclear cells, among which multinucleated giant cells had made their appearance (Fig. 3). These granulomata often distended the lumina of the precapillary branches of the artery and arterioles to many times the original diameter. The elastica was stretched and in the smallest branches was ruptured, permitting the cell mass to move outward (Fig. 4). The surrounding tissue often appeared compressed and pushed aside by the cells as they passed outward from the lumen. Occasional mitotic figures were seen in the large mononuclear cells of the granuloma.

In the animals sacrificed during the first and second weeks after injection, the alterations had progressed further. Cotton fibers lying in the granulomata were carried outward as the cell mass moved through the gap in the elastica and came to lie well beyond the original limits of the vessel wall. At other times, when the elastica ruptured immediately beneath the particle of cotton, the cotton was carried outward at the rounded apex of the granulomatous mass of cells as they pushed from the lumen into the surrounding tissues. This was seen especially well in the precapillary branches. As the cell mass moved outward, a lumen began to appear again in the vessel. Sometimes the granuloma was being covered over by endothelial cells.

Cotton fibers, which had lodged in the portions of the arterial tree where the vessels had thick muscular walls, provoked a granuloma in some instances. At other locations the cotton was being covered by a few endothelial cells. Granulomata occasionally were seen in some of the large branches of the artery, as in an animal sacrificed at the end of 11 days. They partially filled the lumen and were covered by endothelial cells (Fig. 5).

As early as 11 days, the Wilder azocarmine-aniline blue preparations demonstrated numerous fibrils in the granulomata; some of these were argyrophilic, others were stained blue. The argyrophilic fibrils were more abundant in those granulomata that contained mainly large mono-

nuclear cells, and were much less numerous in those composed predominantly of multinucleated giant cells.

Sometimes a cotton fiber became lodged in a muscular artery in such fashion that one end was pointed directly against the wall; in those instances it could be readily seen that the fiber was beginning to penetrate the wall of the vessel.

As early as 4 weeks after injection, granulomata were found that lay entirely outside arterioles and small arteries. The opening through which the granuloma passed to the perivascular tissue had been filled with fibrous tissue in which, in many instances, delicate new elastic fibrils had been laid down. The inner surface of this new tissue closing the gap was covered with endothelium. The lumen of the vessel again was patent (Fig. 6). The passage of the granuloma through the vessel wall was not accompanied by hemorrhage.

Some granulomata were partly within the lumen and partly outside in the surrounding tissue (Figs. 7 and 8). The granuloma then assumed a shape somewhat like that of a dumbbell, with the narrow portion filling the opening in the wall of the arteriole or small artery. In this circumstance, the surface of the portion of the granuloma within the lumen was covered with endothelium. As the granuloma reached the tissue outside the vessel, the cells composing it were less densely packed; it appeared to have expanded. Serial sections often disclosed that granulomata apparently lying outside the vessel still had a small portion plugging a gap in the wall. Also it was obvious that the opening originally had been larger and that after the major portion of the granuloma had passed through, the gap in the wall was being filled in with new fibrous tissue (Figs. 9 and 10).

When the granuloma had formed in a larger artery, it was clearly seen that the cotton fiber was slowly penetrating the wall, often accompanied by a giant cell. Although the elastica interna had ruptured, the granuloma had not yet begun to move outward from the lumen.

Twenty-four animals were allowed to live from 2 to 6 months after injection. Cotton fibers were found in arteries indenting the wall and pushing the elastica interna outward; sometimes these fibers were incompletely surrounded by giant cells, in other instances they had been covered by connective tissue and endothelium. Other cotton fibers near the periphery of the lung were in the alveolar septa, bulging the septal wall inward into the alveolar space, or a pointed end of a fiber projected into the alveolus; a few were found in alveolar spaces, lying within giant cells that also contained anthracotic pigment (Fig. 11). This was

seen as early as 7 weeks after the injection. It was not possible to identify accurately the vessel in which the fiber had lodged because the lumen was reopened and the wall reconstituted after the fiber had escaped.

Numerous granulomata had passed from vessels into the adjacent tissue. As in the animals that were sacrificed in less than 2 months, these granulomata were composed of loosely arranged giant cells. Among them were occasional giant cells holding anthracotic pigment. A few vessels were found in which granulomata had remained in the lumina. These granulomata were reduced chiefly to compact masses of connective tissue which were covered by endothelium and incompletely or completely filled the lumina. Other granulomata had not yet entirely penetrated the vessel wall; a small portion of the cell mass still filled the narrow gap through which the major portion of the granuloma had passed.

Some examples were noted in which a granuloma lay near two arterioles. Each arteriole had a repaired defect in its wall adjacent to the granuloma that appeared to be too large to have been formed in a vessel of that size. It seems reasonable to assume that in these circumstances a small granuloma moved outward from each arteriole and by chance merged to form a single large one.

Restoration of the walls of the vessel, at the gaps through which granulomata had moved to the adjacent tissue or were in the process of escaping, was found in various stages regardless of the length of time the animals were allowed to live (2 to 6 months). There was a growth of connective tissue at the margin of the gap in the vessel wall. This new connective tissue seemingly formed a close-fitting collar about the granuloma as it passed outward; when the last trailing attenuated portion of the granuloma emerged, the gap was closed by connective tissue. Prior to this, endothelium had covered the surface of the hindermost part of the granuloma before it had entered the gap in the vessel wall. Thus the defect was repaired and no blood escaped from the lumen (Figs. 9 and 10).

As early as 2 months after injection certain cells, which had a distinctive appearance and staining reaction, were noted in the connective tissue filling the defect in the vessel wall. These cells resembled in all respects the smooth muscle cells of the media of the vessel (Fig. 12). Mitotic figures have not been found among the muscle cells of the media at the margin of the defect.

Some of the scars in the vessel walls were devoid of elastic tissue;

others contained delicate elastic fibrils (Fig. 6). In yet other vessels, an elastic tissue strand, as heavy as the original elastica interna, had been laid down and often joined the original elastica at a distance from the outwardly turned ends at the site of the rupture in the vessel wall (Fig. 12).

Granulomata occasionally were found in the largest branches of the artery within the lung. They were closely adherent to the intima over a considerable area and were covered with endothelium. In these vessels the granulomatous mass remained within the lumen and underwent organization.

Never did the partial or complete occlusion of the lumen of any of the branches of the pulmonary arteries lead to infarction of the lung.

Discussion

As might have been anticipated, the introduction of cotton fibers through a vein produced foreign body granulomata in branches of the pulmonary arteries. At first, the cells that collected about the particles of cotton resembled in every way large mononuclear wandering cells. These cells most probably were derived from the large mononuclear cells of the blood. Mitotic figures were seen in some of these collections of cells, indicating that cell proliferation was in progress within the granuloma. Very quickly, there appeared multinucleated giant cells among the cells collected about the cotton. The formation of the giant cells was probably similar to that described by Lewis,[1] following the inoculation of tubercle bacilli into hanging-drop preparations of blood.

The granulomata in the precapillary branches of the artery filled and distended the lumen of the vessel. In these branches the granulomata appeared to be under pressure; the elastica was stretched and ruptured within 24 hours. The granuloma then seemed to spring sharply outward into the surrounding tissue and carried with it the cotton fiber or fibers.

Granulomata in the arterioles and medium-sized arteries also distended the lumina of these vessels. The elastica interna was stretched and finally ruptured. It was very obvious that the rupture of the wall of the medium-sized artery occurred in that portion where the media had abruptly become thinner (Figs. 7 to 10).

As to the manner in which the granulomata escape from the vessels, there are two possibilities. The first of these is that the media and adventitia tear following the rupture of the internal elastica; the other possibility is that the cotton fiber penetrates the wall and gradually passes through, the cells of the granuloma following in the wake of the cotton

fiber. Both of these methods occurred. Granulomata were found emerging through a gap in the vessel wall, and the only cotton that could be discovered in serial sections was in the center of the granuloma. Other lesions were seen in which the cotton was penetrating the vessel wall, and cells comprising the granuloma were following it.

Regardless of the manner in which the vessel wall is damaged, once the gap in the wall has be n formed, the granuloma moves outward into the surrounding tissue. One can only surmise what forces the granuloma outward. In the precapillary branches the mass of cells is so crowded together that the lumen is distended, and when the elastica is ruptured, the cells adjacent to the gap emerge through it. The gap, furthermore, is enlarged by the retraction of the elastica when it ruptures.

The passage of the granuloma through the walls of the arteries and arterioles with their muscular media is not so simple as in the precapillary branches. The arteries of medium size and the arterioles are distended by the granulomata. Once a defect is made either by tearing or penetration of the wall by the cotton particles, the cells of the granuloma move into the gap. The granulomata are under the constant pressure of the blood in the pulmonary vascular tree, and, in addition, the heightened pressure during systole probably stretches the hole in the wall and forces the forward portion of the granuloma further into the defect, thus widening and deepening it. When the defect involves all layers of the vessel, the granuloma begins to emerge to the outside and pushes ahead of it the surrounding loose tissue. With each cardiac systole more of the granuloma is forced outward until all of it has emerged from the vessel. Probably the blood pressure within the precapillary branches also plays a part in the passage outward of the granulomata. It is not clear what part the movement of the lung during respiration may play in the process.

Reparative processes begin quickly with the proliferation of fibroblasts. These form a collar about the column of cells of the granuloma and appear to fit snugly about it. Endothelial cells cover the surface of the granuloma and separate it from the circulating blood. When the last portion of the granuloma passes through the intima, the continuity of endothelial surface has been restored. As rapidly as the last part of the granuloma penetrates the media, connective tissue cells fill the gap behind it. When the granuloma finally has passed entirely through the wall, the defect has been filled with connective tissue. It would thus appear that the emergence of the granuloma to the tissues outside the vessel is comparatively slow and affords ample time for restoration of the wall.

The granuloma, or portion of it lying outside the vessel, has a much

less compact structure. The giant cells are more loosely arranged and the cell mass occupies a greater space than it did in the lumen of the vessel. This is very likely due to the fact that it is now no longer confined to a restricted space in the lumen of the vessel nor subjected to the pressure of the blood within the vessel. Also, additional giant cells are formed from the tissue wandering cells that are attracted by the presence of the foreign body. That some of the cells come from the surrounding tissue is evidenced by the anthracotic pigment to be seen in an occasional giant cell; similar pigment has not been found in any cell in a granuloma within the lumen of a vessel except when rupture of the vessel wall has occurred. The presence of anthracotic pigment within the cells in the lumen is a rare occurrence (Fig. 13).

The movement of the cotton fibers does not cease when the granuloma reaches the tissues around the vessel. Long, slender, pointed particles of cotton are to be seen pushing inward the wall of a nearby alveolus, or the naked end of such a fiber projects into the alveolar space. Moreover, tiny fragments of cotton that have escaped through the wall of a precapillary branch of the artery ultimately are found within a giant cell in an alveolus. Probably the constant expansion and contraction of the lung is responsible for the movement of the cotton fibers in the tissue after they have escaped from the vessels.

The compact granuloma within the lumen of the vessel, even in its earliest stages, is impervious to the entrance of blood. Red blood cells and hemosiderin are not found within it. That there is no hemorrhage as the granuloma passes from the vessel is due to the fact that the cell mass acts as a tightly fitting stopper in the defect in the vessel wall. Furthermore, the quick covering of the granuloma with endothelial cells shuts it off from contact with the blood. In addition, the passage of the granuloma to the outside tissues is seemingly slow and reparative processes have sufficient time to keep the margins of the gap tightly closed about the emerging granuloma and to fill the space behind it. After the granulomata have escaped from the vessels, the lumina are again patent.

The restoration of the continuity of the wall of the vessel is complete as soon as the granuloma has passed through it. At first the defect is filled with connective tissue covered with endothelium. Soon delicate elastic fibrils are noted in this scar tissue. Later a more compact strand of elastic tissue is found either incompletely or entirely spanning the area filled with scar tissue. Often this new elastica joins the original ruptured

elastica interna at a distance from its outwardly turned ends. Further changes take place in the scar that fills the defect in the vessel wall. As early as 2 months after injection of the cotton, plump cells having the morphologic and tinctorial properties of smooth muscle cells are distinguishable among the connective tissue elements of the scar. It is not likely that these come from the smooth muscle of the media for no evidences of proliferation of this muscle are to be found. The formation of muscle is most probably due to metaplasia of fibroblasts and is quite analogous to the familiar formation of smooth muscle about the vessels in an organized, canalized thrombus.

The collateral circulation was adequate to prevent infarction of the lungs in the experimental animals; in those instances in which cotton fibers were found in human pulmonary arteries, there was only very slight reduction of the lumina and no infarcts had resulted.

The lack of thrombus formation about the cotton fibers when they lodge within the lumen of the vessel is a striking feature, true in both the experimentally injected animals and in the human cases.

No report of cotton fibers in the pulmonary arteries of the human nor of any experiments in which cotton fibers have been experimentally injected into the venous circulation of animals could be found in the literature. Flory[2] reported the formation of granulomata about cholesterol injected into the pulmonary arterial circulation of rabbits. His experiments were of short duration and no description was given of any movement of the granulomata through the walls of the arteries.

Summary

Cotton fibers accidentally introduced into the pulmonary arterial circulation of the human provoke a foreign body reaction. When cotton fibers are injected into the iliac veins of rats and lodge in the pulmonary arteries, foreign body granulomata are formed. These granulomata in the larger branches of the arteries undergo organization. In arteries of medium size, arterioles, and precapillary branches, the granulomata distend the lumina and often escape through defects in the walls. The defect in the vessel wall is produced by penetration of the cotton fiber through it and also by actual tearing of the wall. The gap is at first filled with fibrous tissue; later some smooth muscle and frequently a new elastica interna are formed. The scar is covered by endothelium. The lumen is again patent after the granuloma has escaped. Some of the cotton fibers ultimately come to lie in alveolar spaces and are surrounded

by foreign body giant cells. There is no thrombosis nor hemorrhage associated with these granulomata, nor does the process lead to infarction of the lungs.

REFERENCES

1. Lewis, M. R. The formation of macrophages, epithelioid cells and giant cells from leucocytes in incubated blood. *Am. J. Path.*, 1925, **1**, 91–100.
2. Flory, C. M. Arterial occlusions produced by emboli from eroded aortic atheromatous plaques. *Am. J. Path.*, 1945, **21**, 549–565.

DESCRIPTION OF PLATES

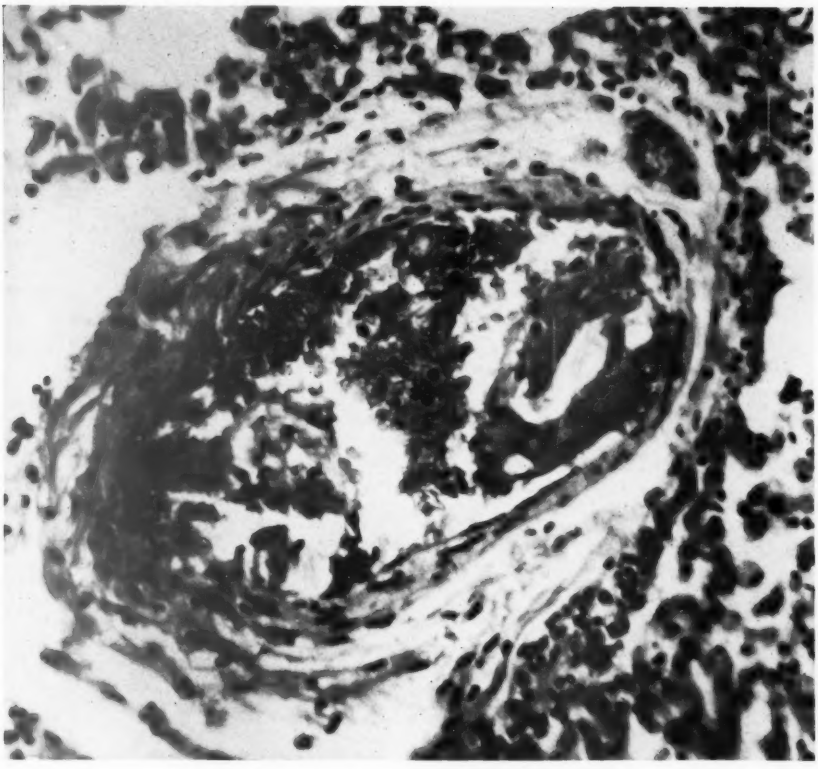
The cotton fibers do not stain. In some of the photomicrographs they show faintly in the granulomata; in others, seemingly empty spaces in the granulomata indicate the size and location of the cotton fibers.

PLATE 86

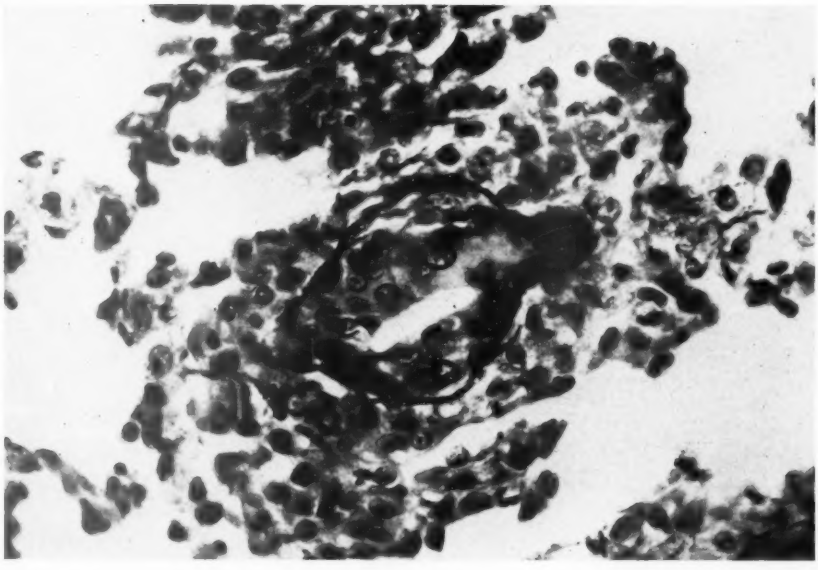
FIG. 1. Cotton fibers in a pulmonary artery of a child, 14 months of age, who had been given a blood transfusion. The cotton is surrounded by giant cells. Hematoxylin and eosin stain. × 390.

FIG. 2. Rat, 24 hours after injection. Cotton fiber in a precapillary branch of a pulmonary artery, surrounded by large mononuclear cells. The vessel is distended, the elastica has ruptured. Hematoxylin and eosin and Weigert's elastic tissue stains. × 660.

1



2



Von Glahn and Hall Pulmonary Emboli of Cotton Fibers

PLATE 87

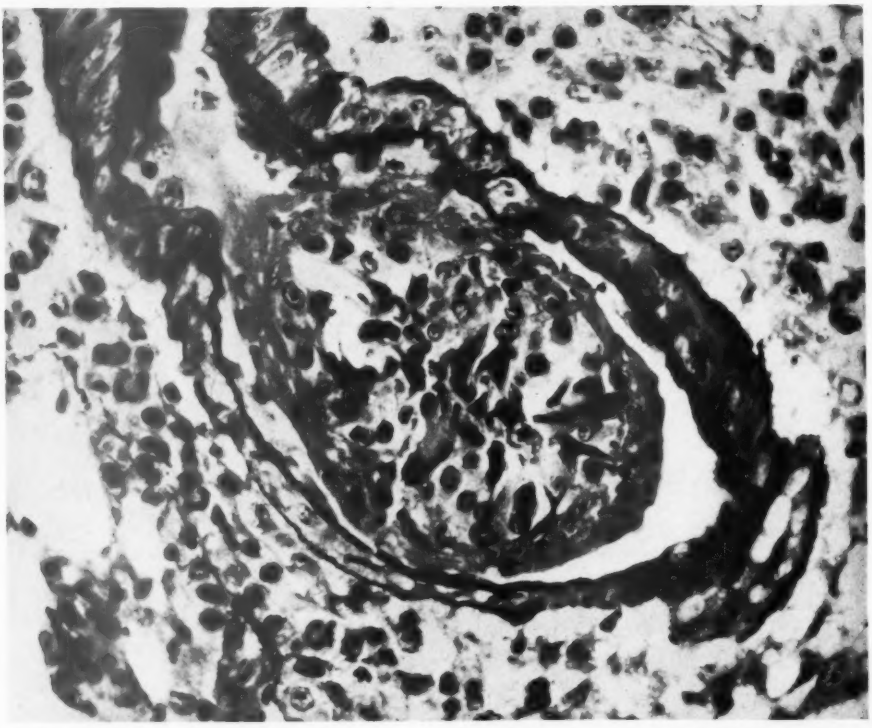
FIG. 3. Rat, 48 hours after injection. Granuloma in a pulmonary artery of medium size. Cotton is seen near the center of the granuloma. The lumen is distended. Hematoxylin and eosin and Weigert's elastic tissue stains. × 660.

FIG. 4. Rat, 48 hours after injection. Granuloma in precapillary branch of pulmonary artery. The granuloma has distended the lumen and is passing outward through a wide gap in the vessel wall, where the elastica has ruptured. A cotton fiber is near the center of the granuloma. Hematoxylin and eosin and Weigert's elastic tissue stains. × 660.

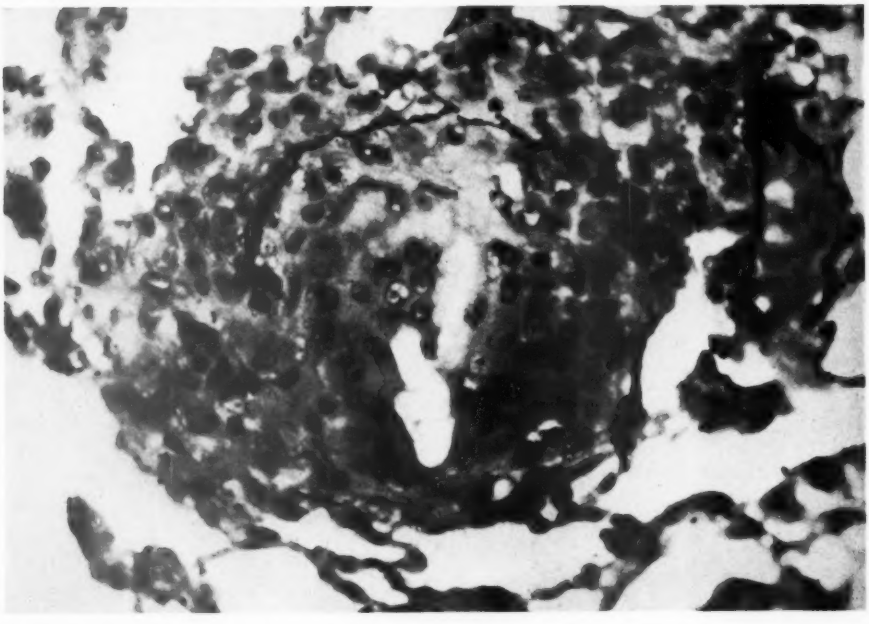
3

4

3



4

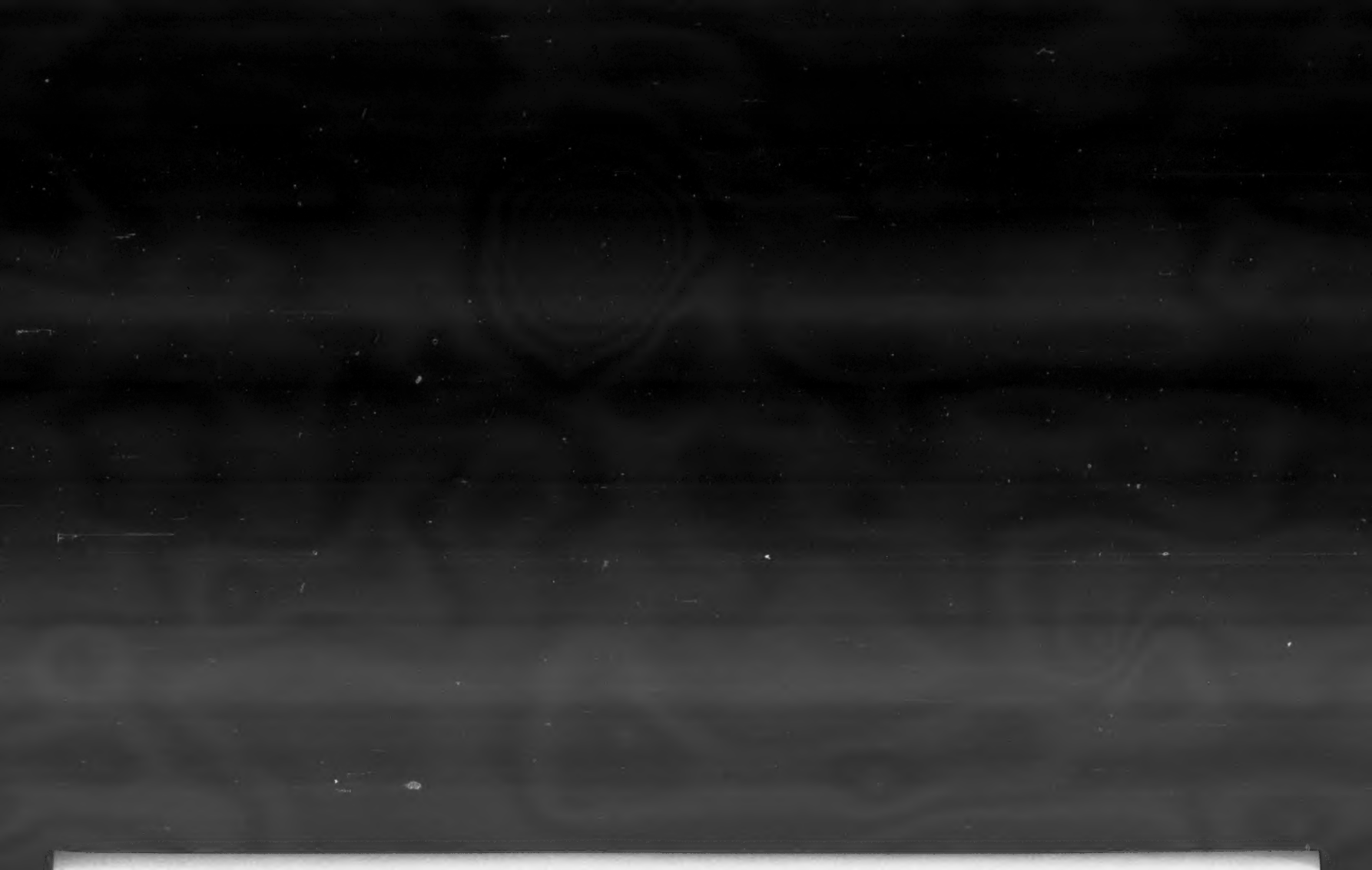


Von Glahn and Hall Pulmonary Emboli of Cotton Fibers

PLATE 88

FIG. 5. Rat, 11 days after injection. Granuloma with many fragments of cotton in large branch of pulmonary artery. The surface of the granuloma is covered by endothelial cells. Hematoxylin and eosin and Weigert's elastic tissue stains. × 190.

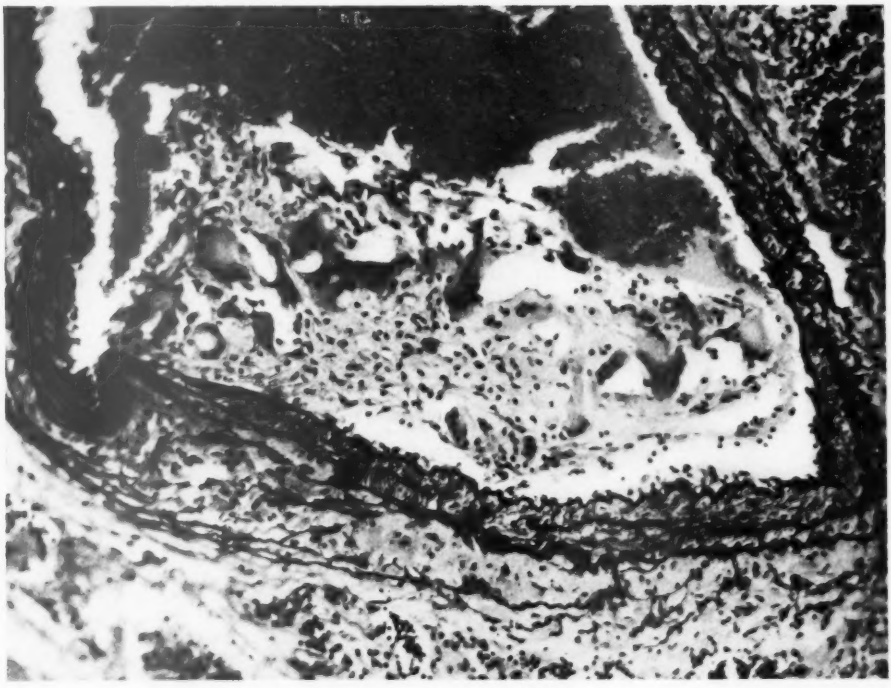
FIG. 6. Rat, 4 weeks after injection. Granuloma lying entirely outside medium-sized branch of pulmonary artery. The defect in the vessel wall is closed by fibrous tissue in which are delicate elastic fibrils. Lumen is again patent. Hematoxylin and eosin and Weigert's elastic tissue stains. × 390.



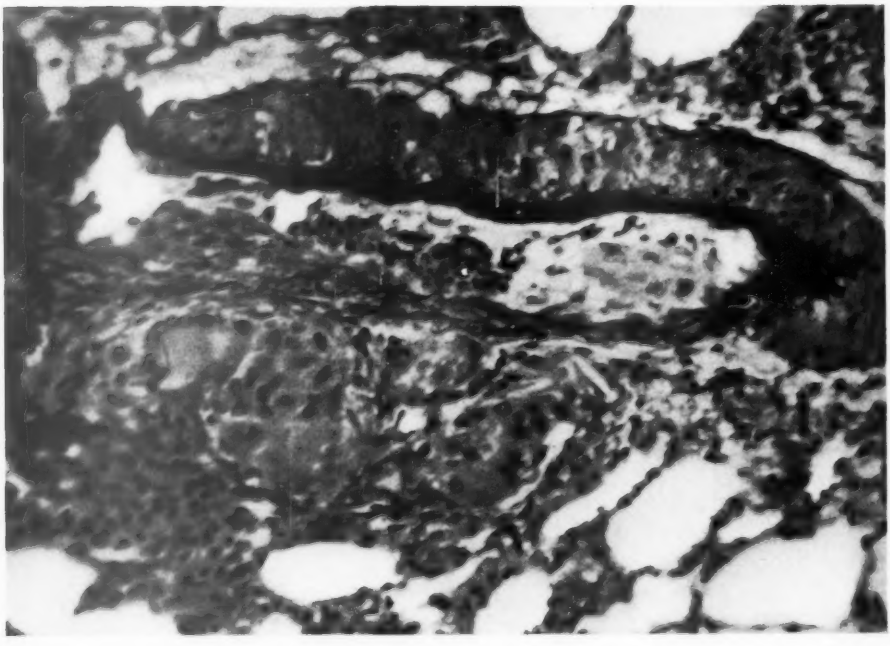
5

6

5



6

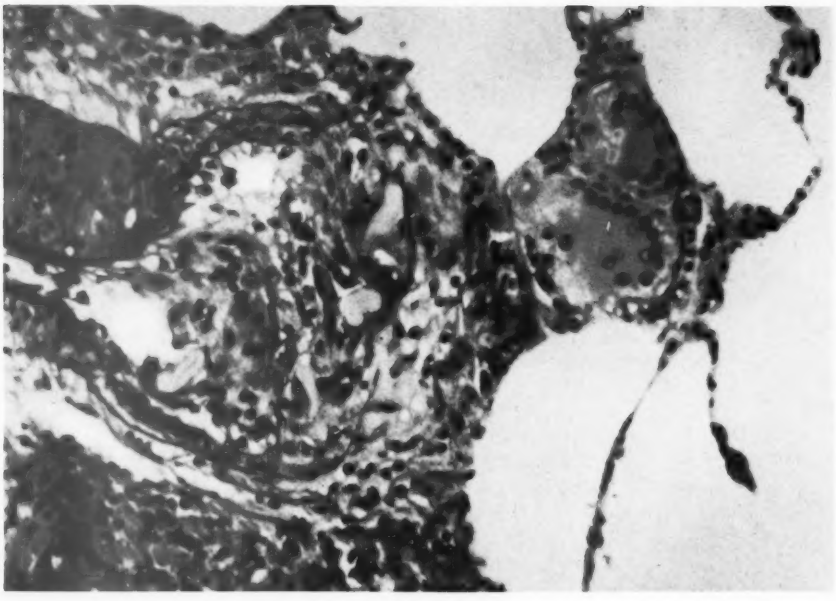


Von Glahn and Hall Pulmonary Emboli of Cotton Fibers

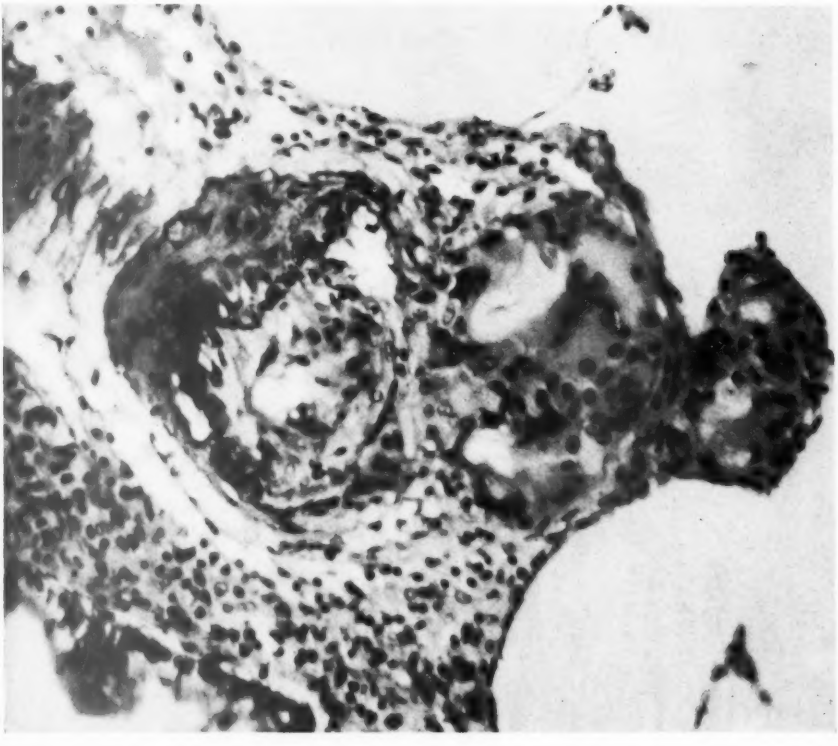
PLATE 89

FIGS. 7 and 8. Rat, 11 days after injection. Granulomata in medium-sized branches of pulmonary artery. The granulomata are passing outward through defects in walls of vessels. In Figure 7 it is to be noted that rupture of the vessel occurred in a normally thinner portion of wall. Hematoxylin and eosin and Weigert's elastic tissue stains. × 390.

7



8



Von Glahn and Hall Pulmonary Emboli of Cotton Fibers

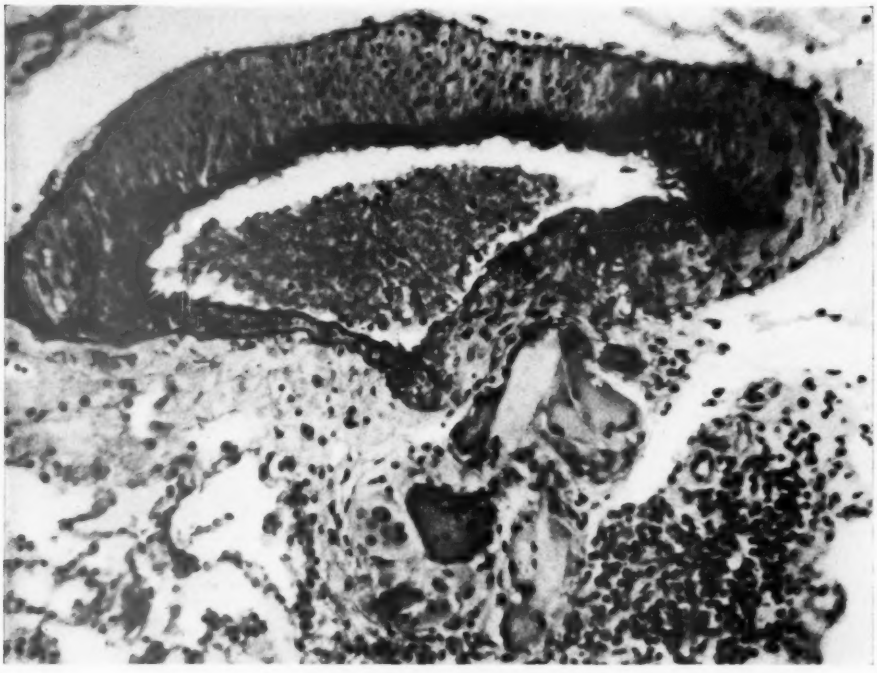
PLATE 90

FIGS. 9 and 10. Rat, 2 months after injection. Medium-sized branch of pulmonary artery. In Figure 9 the granuloma appears to be entirely outside the vessel and the defect in the wall closed completely by fibrous tissue. Figure 10 is from another of the serial sections through the granuloma. The last portion of the granuloma has just emerged from lumen and is covered by endothelium. The rupture occurred in the normally thinner portion of the vessel wall. Hematoxylin and eosin and Weigert's elastic tissue stains. × 280.

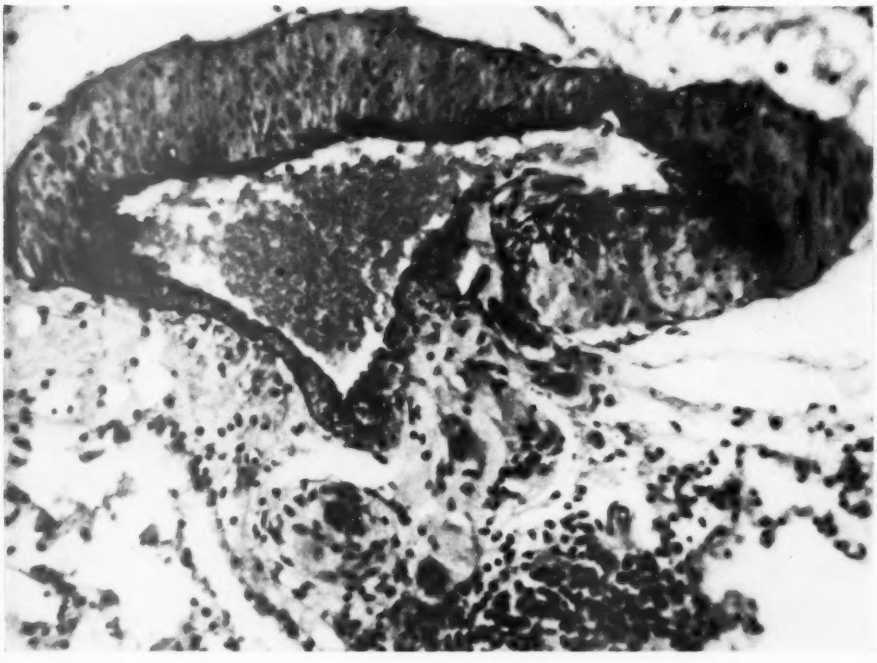
9

10

9



10



Von Glahn and Hall **Pulmonary Emboli of Cotton Fibers**

PLATE 91

FIG. 11. Rat, 2 months after injection. A small piece of cotton in an alveolus. The cotton is surrounded by a giant cell containing anthracotic pigment. In a nearby septum there are two pieces of cotton, each with a giant cell, near two precapillary branches of an artery. Hematoxylin and eosin stain. × 588.

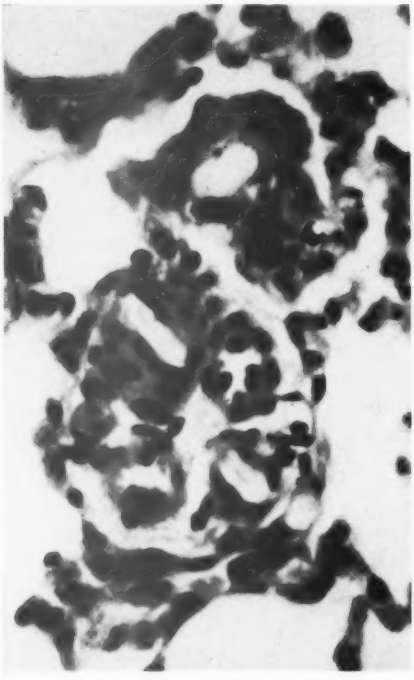
FIG. 12. Rat, 3 months after injection. Granuloma lying entirely outside medium-sized artery. A defect in the wall of the artery is filled by fibrous tissue in which are cells identical with smooth muscle. New elastic tissue joins the ruptured original elastica at a distance from the outwardly turned ends. Rupture occurred through a normally thinner portion of vessel wall. Hematoxylin and eosin and Weigert's elastic tissue stains. × 588.

FIG. 13. Rat, 8 days after injection. Granuloma in a precapillary branch of artery. The lumen is greatly distended. The elastica has ruptured in two places and the granuloma is escaping through a wide gap in the vessel wall. Anthracotic pigment occurs in two of the giant cells. Hematoxylin and eosin and Weigert's elastic tissue stains. × 588.

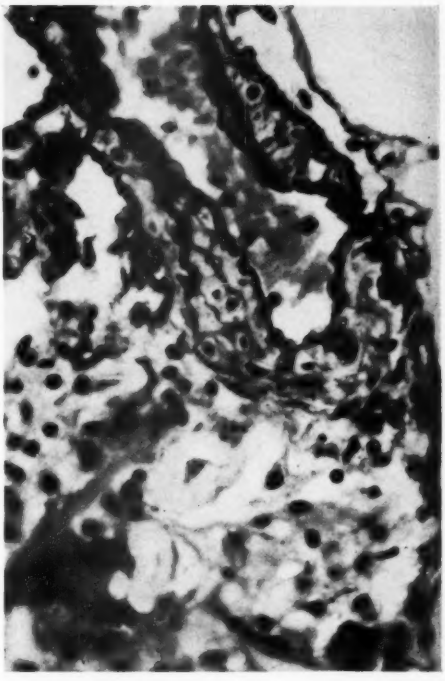
11

13

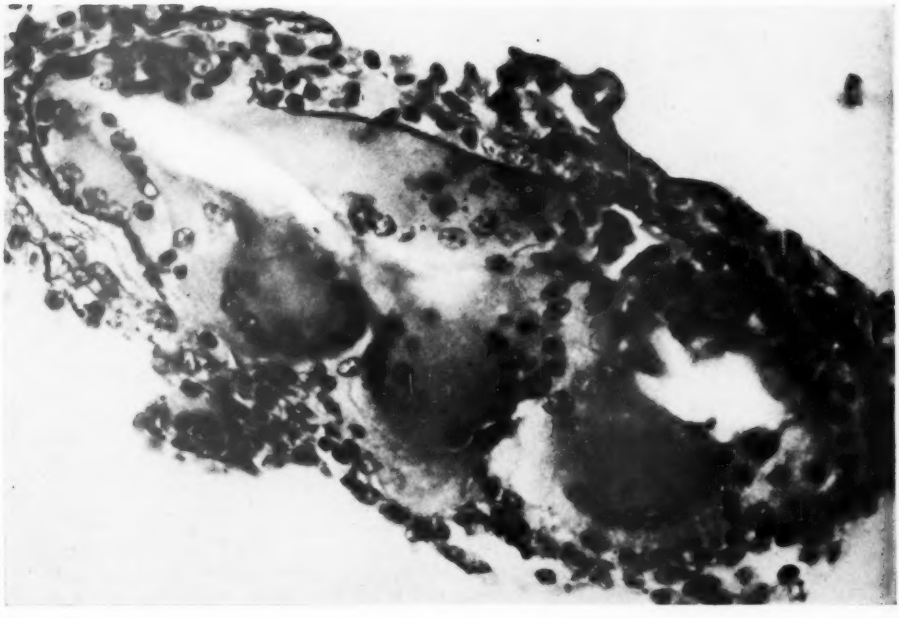
11



1



13



HISTOCHEMICAL STUDIES IN GAUCHER'S DISEASE*

ROGER W. MORRISON, M.D., and MARVIN H. HACK
(From the Department of Pathology, University of Illinois College of Medicine, Chicago, Ill.)

In Gaucher's disease there are many large foamy cells in the spleen and liver, and often in the bone marrow and lymph nodes.[1,2] Most pathologists now agree that these cells arise from the reticulum and from histiocytes in the spleen, liver, lymph nodes, and bone marrow.[1,2] Several investigators have shown that kerasin, a cerebroside, is accumulated in Gaucher's disease.[3-6]

The use of histochemical methods to identify and localize kerasin in sections of spleens from patients with Gaucher's disease seemed worthy of trial. The physical properties of kerasin are of importance in such an approach. It is soluble in warm (65° C.) methyl alcohol and chloroform (1:1), in warm (65° C.) ethyl alcohol, and in pyridine. It is insoluble in cold methyl alcohol, ethyl alcohol, xylene, ether, petroleum ether, and acetone. An ultraviolet absorption peak for kerasin has been reported at 2675 to 2700 Angstrom units.[7]

Kerasin is composed of lignoceric acid, sphingosine, and a hexose, usually galactose,[3] but sometimes glucose. With a compound of this nature the periodic acid-leukofuchsin method[8,9] might be expected to produce a positive reaction. In that case one might expect the hexose to be oxidized by the periodic acid to a diglycollic aldehyde. When this reacts with the fuchsin-sulfurous acid reagent, it forms the corresponding colored (rose-purple) addition product. This was confirmed by means of a spot test with pure kerasin isolated from human brain. In sections the altered carbohydrate remains bound to the insoluble components of kerasin and enables the colored product to be accurately localized microscopically.

METHODS AND OBSERVATIONS

Spleens† removed from 3 patients with Gaucher's disease were available for study. A block from each spleen was fixed in 10 per cent formalin. A second block was taken from one fresh specimen and fixed by freezing and drying. Paraffin sections from all four blocks were passed through xylene, absolute alcohol, and 70 per cent alcohol at room temperature. The sections were then treated for reactive carbohydrate groups by Hotchkiss'[9] method.

* Aided in part by a grant from the American Cancer Society, recommended by the Committee on Growth, National Research Council.
Received for publication, June 24, 1948.

† Drs. Paul Klemperer and Otto Saphir each very kindly furnished material from one of his cases of Gaucher's disease.

On microscopic examination a brilliant rose-purple color was seen in the Gaucher cells (Figs. 1 and 2). The results seemed equally good with formalin-fixed or frozen-dried material. The red pulp (Fig. 1) was mostly replaced by irregularly shaped spaces containing many large, oval, rose-purple Gaucher cells. The reticulum fibers of the spleen stood out boldly. No rose-purple cells were seen in the centers of the lymphoid follicles, but toward the periphery a few small ones were noted (Fig. 2). Surrounding the lymphoid follicles were masses of brilliantly colored Gaucher cells. These showed the characteristic eccentric position of the nucleus which itself was free of the colored compound. The cytoplasm was packed with granules of rose-purple material.

An attempt was made to extract the reactive material from the cells using solvents known to dissolve kerasin. Sections from the four blocks were deparaffinized and successively immersed in warm (65° C.) methyl alcohol-chloroform mixture (1:1) for 12 hours and warm ethyl alcohol for 12 hours. They were then stained as before. The Gaucher cells showed as vivid a color reaction as in the unextracted sections. The question then arose whether kerasin can be extracted from sections after fixation with formalin or by freezing and drying.

Accordingly, an experiment was arranged in which one white rat was given intravenously 25 mg. of purified kerasin prepared from human brains and each of 4 rats was given 100 mg. intraperitoneally. Autopsies were performed after 20 minutes, and after 4, 8, 24, and 72 hours, respectively. Two blocks were taken from the liver of each animal; one was fixed in 10 per cent formalin and the other by freezing and drying. Paraffin sections from all blocks were treated by the periodic acid-leukofuchsin method, avoiding the use of hot solvents. Many uniformly rose-purple, discrete, highly refractile globules about 1 or 2 μ in diameter were seen in the cytoplasm of the Kupffer cells. These globules were thought to be kerasin. Their specific location in Kupffer cells distinguished them from glycoprotein granules seen in the cytoplasm of many different cells. Glycoprotein granules were not refractile, varied much in size, took the rose-purple color with varying intensity, and had irregular fuzzy outlines.

Extraction of the material believed to be kerasin was undertaken. Deparaffinized sections from the same blocks of rats' livers were placed in warm (65° C.) methyl alcohol and chloroform solution for 12 hours. After extraction they were treated by the periodic acid-leukofuchsin method. Examination disclosed a marked decrease in the number of globules (presumably kerasin) as well as a marked reduction in the

number of Kupffer cells containing globules. The glycoprotein granules in these sections remained unaltered. The observed differences in extracted and unextracted sections were less marked in the 2 animals killed 24 and 72 hours after injection than in the other animals. Sections of liver from a control animal showed no refractile globules in the Kupffer cells. Exposure of deparaffinized sections to a warm (65° C.) methyl alcohol and chloroform mixture produced no detectable histologic alterations.

A formalin-fixed deparaffinized section from each of the 3 Gaucher's spleens and a frozen-dried deparaffinized section from one fresh specimen were examined with the ultraviolet microscope. The Gaucher cells in all sections almost completely absorbed ultraviolet light at 2800 Angstrom units. On the other hand, there was no peak absorption band when purified kerasin was exposed to ultraviolet light.

A small amount of formalin-fixed material from the spleen of a patient with Niemann-Pick's disease was available for study. Deparaffinized formalin-fixed sections were treated by the periodic acid-leukofuchsin method. None of the vast numbers of typical foam cells in the pulp showed the rose-purple color.

Discussion

As anticipated, purified kerasin gives a rose-purple color when subjected to the periodic acid-leukofuchsin method. The galactose in the kerasin probably provides the adjacent hydroxyl groups requisite for oxidation to aldehyde by periodic acid.

The appearance of the rose-purple color in the sections from human Gaucher spleens demonstrates that kerasin may be present and localized within the Gaucher cells. Heretofore the identification of kerasin has been by macrochemical extraction from large portions of spleen. The inference was then made that the abnormal substance (kerasin) was present in the multitudes of foam cells seen microscopically. Our evidence is the most specific yet advanced to show that kerasin occurs within the Gaucher cell.

Kerasin apparently was not extracted from the sections of Gaucher's spleens exposed to a mixture of warm methyl alcohol and chloroform. When kerasin is injected into rats it appears in the Kupffer cells of the liver as particulate material. With these solvents it can be removed readily from sections of frozen-dried or formalin-fixed tissue. Decreased amounts were extracted from tissues of animals living 24 and 72 hours. A possible explanation is that metabolic processes might have fixed the

splenic kerasin in place by uniting it with another substance. The idea that protein and cerebroside might be linked is attractive. Such a linkage, if demonstrated, might explain the failure to extract kerasin with the appropriate solvents. On the other hand, there is little chemical evidence for the existence of such a lipid-protein complex.

Ultraviolet absorption studies support the idea that a protein is present in the Gaucher cell. Dworacek and Pesta[7] reported that maximum absorption for kerasin occurred between 2675 to 2700 Angstrom units. Using purified kerasin, it was not possible to confirm their findings; *i.e.*, in the range 2300 to 3300 Angstrom units, kerasin was found to have no region of maximal absorption.

Under the ultraviolet microscope, Gaucher cells showed a strong absorption band at 2800 Angstrom units. This indicates the presence of large amounts of protein in Gaucher cells, for it is well known that proteins absorb ultraviolet light of 2800 Angstrom units. It is possible, but not proved, that this protein and kerasin are joined together.

Figure 1 shows the striking reaction of the reticulum to the periodic acid-leukofuchsin method. This is due to the high content of reactive material in the reticulum fibers. The polysaccharide bound to the protein reacts in the same manner as does the kerasin. The color of the glycoprotein granules within the cells in rats' livers can be explained on the same basis.

The observation, illustrated in Figure 2, that the cells contain more rose-purple material as they approach the periphery of the lymphoid follicle is of interest. It is possible that here both lymphocytes and Gaucher cells are seen arising from the primitive cells, offspring of the reticulum of the lymphoid follicle. This can be regarded as further evidence that Gaucher cells arise from the reticulum. It also strengthens the belief that in Gaucher's disease the fault lies within the cell involved, that is, the Gaucher cell is not a phagocyte collecting kerasin from the passing stream. It is probable that as the cell matures, the inherent metabolic error becomes apparent.

The material from the case of Niemann-Pick's disease illustrated the differential diagnostic possibilities of the periodic acid-leukofuchsin method. The pathognomonic lipid in this disease is sphingomyelin.[2,3] Its molecule does not include a hexose. In agreement with this, the rose-purple color failed to appear when the method was tried on pure sphingomyelin and on tissue from Niemann-Pick's disease. The method would seem applicable to smears of material collected at sternal puncture in which the presence of large cells with abundant foamy purple-red cyto-

plasm would certainly bring Gaucher's disease into consideration. In this manner it is possible histologically to differentiate between Gaucher's disease and Niemann-Pick's disease.

Conclusions

It has been demonstrated that kerasin, because of its hexose component, gives the characteristic rose-purple response with the periodic acid-leukofuchsin method.

For the first time kerasin has been localized within the Gaucher cell. Failure to extract kerasin from the cell suggests that it may be present in combination with some other substance.

The periodic acid-leukofuchsin method has differential diagnostic possibilities for the lipidic diseases. Whereas Gaucher cells develop an intense rose-purple, the characteristic foam cells of Niemann-Pick's disease remain colorless with this procedure.

REFERENCES

1. MacCallum, W. G. A Textbook of Pathology. W. B. Saunders Co., Philadelphia & London, 1940, ed. 7, p. 1022.

2. Thannhauser, S. J. Lipidoses: Diseases of the Cellular Lipid Metabolism. Oxford University Press, New York, 1940, 370 pp.

3. Thierfelder, H., and Klenk, E. Die Chemie der Cerebroside und Phosphatide. J. Springer, Berlin, 1930, 224 pp.

4. Lieb, H. Cerebrosidspeicherung bei Splenomegalie, Typus Gaucher. *Ztschr. f. physiol. Chem.*, 1924, **140**, 305–313.

5. Cushing, E. H., and Stout, A. P. Gaucher's disease, with report of a case showing bone disintegration and joint involvement. *Arch. Surg.*, 1926, **12**, 539–560.

6. Bloom, W., and Kern, R. Spleens from Gaucher's disease and lipoid-histiocytosis. The chemical analysis. *Arch. Int. Med.*, 1927, **39**, 456–461.

7. Dworacek, E., and Pesta, H. Ueber einen spektralanalytischen Nachweis des Kerasins und experimentelle Cerebrosidspeicherung im Sinne eines Morbus Gaucher. *Wien. klin. Wchnschr.*, 1939, **52**, 332–337.

8. McManus, J. F. A. Histological demonstration of mucin after periodic acid. *Nature, London*, 1946, **158**, 202.

9. Hotchkiss, R. D. A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. *Arch. Biochem.*, 1948, **16**, 131–141.

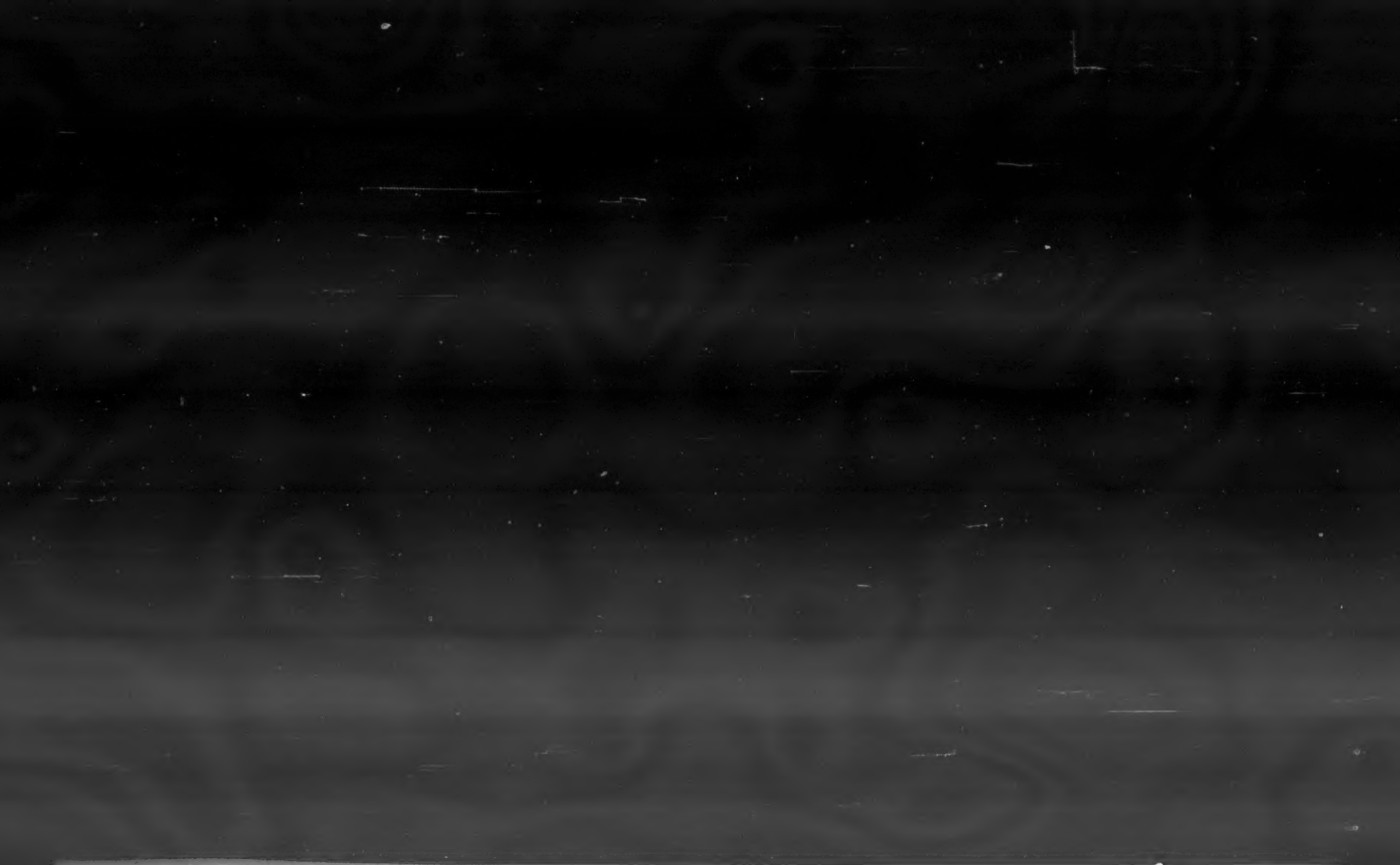
[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 92

FIG. 1. Spleen, Gaucher's disease. The pulp is mostly replaced by irregularly shaped spaces containing many large, oval, Gaucher cells. The reticulum is clearly shown. Formalin (10 per cent) fixation. Periodic acid-leukofuchsin method of staining. × 240.

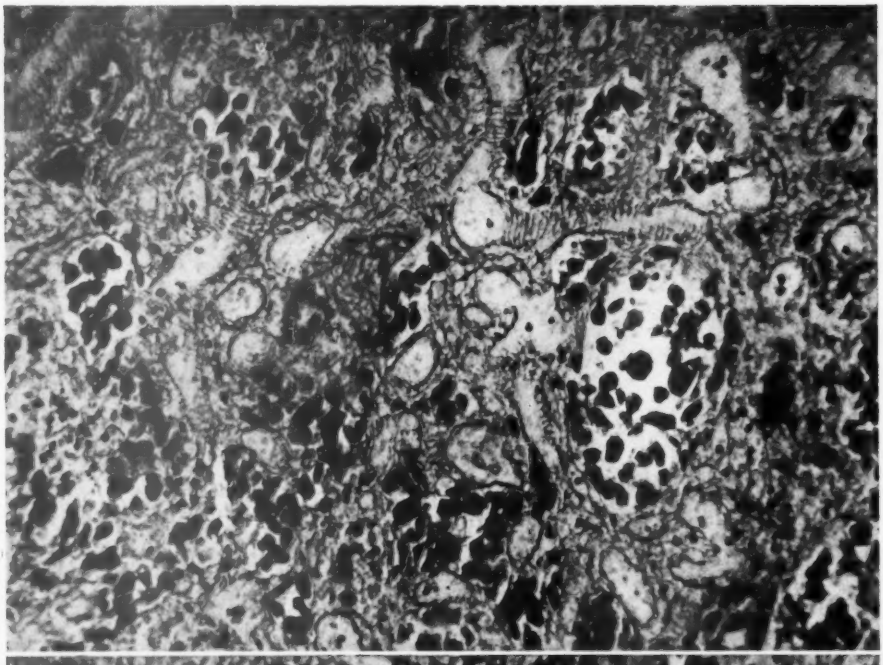
FIG. 2. Spleen, Gaucher's disease. The cells contain more periodic acid-leukofuchsin reacting material as they approach the periphery of the lymphoid follicle. Formalin (10 per cent) fixation. Periodic acid-leukofuchsin method of staining. × 240.



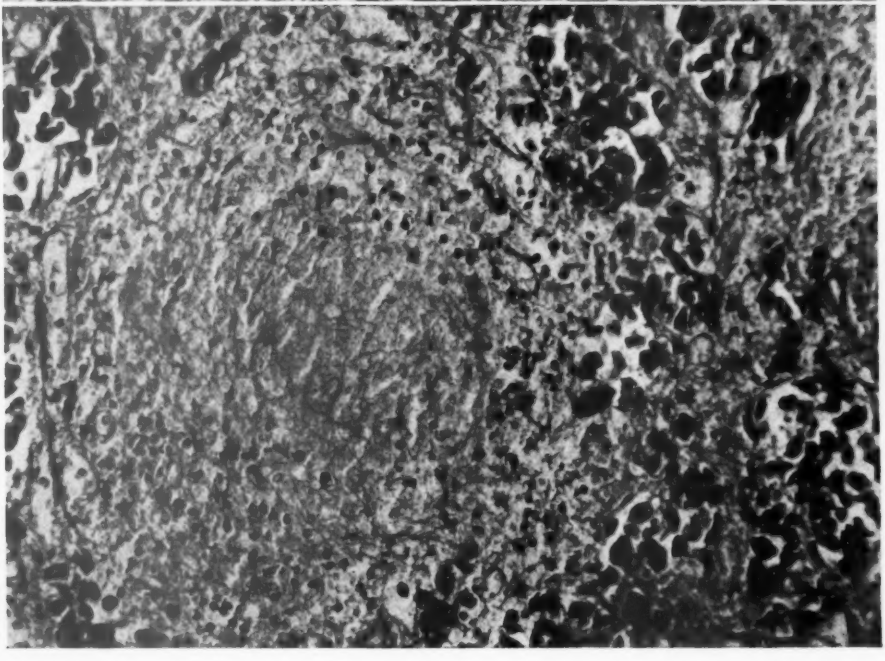
1

2

1



2



A TRANSPLANTABLE SPLENIC TUMOR RICH IN MAST CELLS
OBSERVATIONS ON MAST CELLS IN VARIED NEOPLASMS*

TALIA BALL, M.D.,† and JACOB FURTH, M.D.
(From the Branch 10 Laboratories of Veterans Administration Hospital and Southwestern Medical College, Dallas, Texas)

Numerous investigators have noted a mast cell increment in and about many tumors.[1,2] Many recent studies have suggested a relation of mast cells to heparin formation.[3-5] Hyperheparinemia has been found to be the cause of certain hemorrhagic tendencies.[6]

The finding of a transplantable splenic tumor of mice, characteristically rich in mast cells, called our attention to problems related to these cells. Indeed, the question arose whether the cells of these splenic neoplasms themselves are related to mast cells. The predominant cell type of these splenic tumors[7] is a polygonal epithelium-like cell, thought to be a neoplastic derivative of splenic reticulum. It will hereafter be referred to as the ER (epithelium-like reticulum) cell. For the study already reported, special stains showing mast cells were not employed and the richness of these neoplasms in mast cells was overlooked. Subsequently, sections from the spontaneous neoplasms of this type previously reported have been restained for mast cells and it has been found that their presence in these ER tumors is characteristic.

Histologic studies were undertaken to determine the relation of these two types of cells, to find out how frequently and characteristically neoplasms of mice of this and other types contain mast cells, and to obtain information on the histogenesis of mast cells.

MATERIAL AND METHODS

Material. Sections from the following neoplasms of mice, fixed in Zenker's formol solution, were stained with Giemsa's stain for mast cells: 18 spontaneous benign ER splenic tumors; 4 other splenic tumors; 6 transplanted ER splenic tumors; 7 miscellaneous spontaneous carcinomas; 12 miscellaneous sarcomas (3 of which were transplanted, 2 induced, and 7 spontaneous); 9 transplanted carcinomas; 44 spontaneous leukemias; 15 spontaneous pulmonary adenomas; 3 transplanted endotheliomas; and 162 varied transplanted ovarian tumors.

Fixation. Zenker's-formol mixture seemed adequate for fixation. Apparently the granules were as easily demonstrable in routine autopsies made up to 24 hours after death as in tissues fixed immediately after death. Thionin blue stains, made on several imprints and on a few

* Received for publication, July 16, 1948.
† Visiting Fellow in Experimental Pathology of the University of Istanbul.

sections, did not demonstrate the mast cell granules better than Giemsa's stain, and the latter gave a superior staining of the general background. Routine hematoxylin and eosin sections seldom disclosed mast cells.

Transplantation. Transplantation of these splenic tumors, reported here for the first time, was attempted by cutting up the tumor in Tyrode's solution and injecting or implanting the tumor fragments in the subcutaneous tissue or spleen, in the anterior chamber of the eye, or intravenously. Numerous animals were irradiated with approximately 400 r. either before or shortly after injection to increase their susceptibility to the growth, the site of injection being shielded. Because of the chronicity of the tumors the irradiation was repeated in several instances. The factors of irradiation were: 400 r.: 140 kv., 5 ma.; filter, 1 mm. Al; target distance, 28 cm.; time, 5 minutes, 38 seconds. 200 r.: 140 kv., 5 ma.; filter, 1 mm. Al; target distance, 28 cm.; time, 2 minutes, 49 seconds. 350 r.: 140 kv., 5 ma.; filter, 1 mm. Al; target distance, 28 cm.; time, 4 minutes, 58 seconds.

Morphologic Characteristics of ER Splenic Tumors

These spontaneous neoplasms usually were single and circumscribed but not encapsulated, ranging from 4 to 22 mm. in greatest diameter. They were gray-white, soft, and frequently spotted with areas of hemorrhage or necrosis.[7] Erythropoietic foci were frequent in these tumors (Fig. 1). The neoplastic cells were polygonal with finely granular, slightly acidophilic cytoplasm and a large vesicular nucleus containing one or two nucleoli (Figs. 1 and 5). The predominant cells were unlike the usual reticulum cells hitherto described in man and in animals. They are designated as "ER" cells only for expediency and in the belief that their most likely precursor is the reticular framework cell of the spleen.

In hematoxylin and eosin preparations numerous dark-staining polymorphous cells were noted in and about reticular cells. These resembled Kupffer cells, and when stained with Giemsa's stain were found to be loaded with granules as seen in Figure 2. All but one of the 18 spontaneous splenic tumors of this type which were examined contained numerous mast cells.

The size of the mast cells in the tumors varied from about 8 by 8 μ to about 11 by 30 μ with an average diameter of about 11.5 μ. The mast cells were in contact with tumor cells. Some were in the center of a group of tumor cells while others were at the periphery of such groups, with cytoplasmic projections extending between the tumor cells. At the periphery, some were located between the tumor cells and capillaries and only

rarely could they be seen in the capillaries. They also were in the "capsule" of the tumor and were abundant about the connective tissue stroma and about the adventitia of the vessels.

The shape of the mast cells was variable: round, oval, pear-shaped, elongated, or ramifying. The nuclei usually were oval, sometimes round. The nuclear-cytoplasmic ratio was approximately 1 to 1½. The chromatin of nuclei of mast cells usually was dense and took on a homogenous blue color with Giemsa's solution (Figs. 3 and 8). The nuclei of ER cells were larger, always nearly round; the chromatin structure was loose and there were one or two nucleoli.

The metachromatic granules of the mast cells were coarse, although finer than those of normal mast cells, and usually so dense that the nucleus was barely detectable. However, when the sections were understained, it was possible to see clearly the blue-colored nuclei amidst the violet-colored granules (Figs. 3 and 8). Rarely, an unstained zone could be seen immediately around the nucleus where the granules were lacking. Since no special precautions were taken to preserve the granules before fixation or during the process of staining, no special significance can be attached to differences in density of granules.

In general mast cells were not connected with one another. In some places they appeared to make contact by cytoplasmic extensions (Fig. 8) and they occurred frequently in groups. Mitotic figures were not noted among them but rarely binucleated forms occurred (Fig. 8). The latter were suggestive of amitotic division. Occasionally a spherical body with size, shape, and staining qualities of a red cell was seen in the cytoplasm of a mast cell; or a vacuole was noted in similar location.

The distribution of mast cells in spontaneous splenic tumors is summarized in Table I.

TABLE I

Distribution of Mast Cells in Spontaneous ER Splenic Tumors

Mast cell distribution	No. of mice	Central parts	Peripheral parts	Connective tissue stroma	Perivascular area	Perihemorrhagic area
Uneven	10	13	17	19	17	18
Even	7	13	13	13	13	13

In the last five columns the figures indicate the number of mast cells per 0.3 sq. mm. area.

In 7 tumors mast cells were evenly distributed; in 10 the number varied in different parts of the tumor. The greatest density was noted in the peripheral region and about the heavy strands of connective tissue stroma traversing the tumor. In some parts of the tumor, notably about

hemorrhages, mast cells usually were abundant. They were rare in the splenic tissue surrounding the tumor and also rare in the normal portions of the spleen. In only one mouse with this type of tumor was the red pulp full of mast cells as described by Deringer and Dunn[8] in a study of mast-celled growths in mice.

Mouse Akh 152 died at 8 months of age and had a tumor 1.5 cm. in diameter in an otherwise normal spleen. The tumor was gray with brown-red areas of hemorrhage and necrosis. Its microscopic appearance was typical of an ER neoplasm with large numbers of mast cells. At the periphery of the tumor there were dense collections of mast cells. The pulp of the spleen contained many mast cells in circumscribed foci. Binucleated forms were numerous and chain formations infrequent. Mast cells in the spleen were somewhat larger (averaging about 14 by 11.6 μ) than those in the tumor, averaging about 11 by 11 μ. Almost every mast cell exhibited an unstained halo around the nucleus. The bone marrow contained numerous foci of mast cells with several chain formations (Fig. 4). Other organs from this animal were not available for microscopic study.

Only one of the 18 spontaneous ER growths that had been studied microscopically was free from mast cells.

At 11 months of age, mouse 2477 had a spherical gray-white splenic tumor, 1.5 cm. in greatest diameter. The spleen itself measured only 0.5 by 1.5 cm. The neoplastic cells were like those in the tumors already described. Figure 5 shows epithelium-like masses of these cells, in places with reticular arrangements. There were large numbers of hemopoietic cells in the spleen, predominantly erythroblasts, but no mast cells.

The ER cells of transplanted tumors were similar in appearance to those of spontaneous tumors (Figs. 6 to 9), and the relation of mast cells to tumor cells in transplanted neoplasms was the same as in the spontaneous neoplasms. The difference in the mast cell density between peripheral and central parts of the tumor was more marked in the transplanted than in the spontaneous tumors.

Transplantation of ER (Epithelium-Like, Reticulum-Celled) Splenic Tumors

Because of the unusual histologic character of the ER splenic tumor and its constant relation to mast cells, repeated efforts have been made during the past 8 years to secure a readily transplantable tumor of this type. This growth has thus far been encountered only in Ak mice and their hybrids, and in man.[7] Of six attempts at transplantation of spontaneous ER tumors, three were successful. The results of the transplantation experiments are summarized in Table II.

There were two interesting features in these transplantation studies: The unusually slow growth of this tumor, which has not changed in the course of several successive passages; and the adherence to the charac-

TABLE II

Attempted Implantations of Spontaneous ER Splenic Tumors

Donor no.	No. of Passages	No. of Subpassages	Route of transplantation	Treatment of recipient	Result of transplantations‡ Female	Result of transplantations‡ Male	Latent period
							months
1147	11	5	Subcutaneous		33/1	72/11	
			Subcutaneous	Pre-irradiated		12/2	2–8
			Intrasplenic		2/0	7/0	
			Intrasplenic	Pre-irradiated	3/0	3/0	3–4
			Intra-ocular			5/0	
			Intravenous			8/0	
464	5	3	Subcutaneous		16/9	3/2	4–6
			Subcutaneous	Pre-irradiated	5/0	1/1	5
			Subcutaneous*			9/3	
300		1	Subcutaneous†		9/2		4.5–6
			Subcutaneous		4/0	8/0	
495	0	0	Subcutaneous*		3/0	12/0	
39	0	0	Subcutaneous†		3/0	3/0	
			Subcutaneous*		3/0	3/0	
2477	0	0	Intrasplenic			2/0	
			Intrasplenic	Castrated	2/0	1/0	
			Subcutaneous		1/0	2/0	
			Subcutaneous	Castrated	2/0		

* and intraperitoneal.
† and intraperitoneal and intravenous.
‡ The figures in these two columns indicate the number of mice injected over the number of positives.

teristic cell types, namely, to epithelium-like reticulum cells with intervening mast cells.

The latent period, which is the interval between implantation of fragments of the tumor into the subcutaneous tissue and the appearance of a palpable nodule, varied between 2 and 8 months and averaged about 5 months. For example, in passage IIb of strain 1147, one mouse had a palpable tumor 52 days after injection, another only at 165 days. The latter tumor was excised at 281 days when it measured 2 by 2 by 1 cm. The transplanted tumors were invariably slow-growing and the animals had to be sacrificed when the tumor measured 1 to 2 cm. in greatest diameter because of the liability of the host animals to develop leukemia. Metastases have never been observed and removed tumors did not recur.

Normal males seemed slightly more susceptible (17 per cent) than normal females (10 per cent) to subcutaneous implantation of the tumor. Attempts at transmission by routes other than subcutaneous (intravenous, intrasplenic, and intra-ocular routes) made in a small number of mice were unsuccessful.

The difficulties of carrying on such strains by successive transplantations are indicated in Table II. Only the first strain listed is still alive, and five successive passages of this strain failed to increase the percentage of successful transplantations or to shorten the period of latency. Pre-irradiation did not enhance susceptibility to these neoplasms as it did to many others.

Absence of Mast Cells in Four Splenic Tumors Other than the Described ER Type

Four splenic neoplasms other than the type described were studied for comparison. None of them contained mast cells. One was fibroma-like; the other three resembled monocytoma or histocytoma. One of the latter was in an animal which had neoplastic cells in the liver and another in a mouse with generalized leukemia.

Mast Cells in and about Miscellaneous Neoplasms

Neoplasms in general are free from mast cells. Luteoma is an exception (Table III). In some luteomas the number of mast cells exceeded that in the ER tumors (Fig. 10). None of the epithelial tumors examined contained them but they were encountered in several sarcomas and in an epithelioma.

The accumulation of mast cells around a tumor is more common. As Table III shows, they were frequent about some granulosa cell tumors and about some carcinomas and sarcomas. The transplanted granulosa cell tumors of strain I, about which no mast cells were seen, were of the folliculoid type. Tumors of strain IV had a diffuse pattern and those of strain V had areas of both diffuse and folliculoid types. The behavior of the mixed strain XI was noteworthy. This strain had lines with tumors of either or both granulosa and lutein cells. The mast cells invariably surrounded the granulosa cell masses but not the lutein cells.

Two of the carcinomas about which mast cells accumulated originated in the mammary gland. Those with no mast cells were 3 epidermoid carcinomas of the skin and 1 lacrymal gland carcinoma. The transplanted carcinomas with no mast cells were anaplastic, derived from endometrium. The 2 leukemias with a few mast cells about the leukemic infiltrations were monocytic; other monocytic, lymphoid, and myeloid leukemias did not contain mast cells. The endotheliomas were transplanted growths with rich capillary formations containing red blood corpuscles. One of the thymic sarcomas was spontaneous, the others were transplanted; the mast cells were found in the latter. The other

sarcomas were: 2 liposarcomas, 3 fibrosarcomas, 1 methylcholanthrene-induced and 1 benzpyrene-induced fibrosarcoma.

TABLE III

Mast Cells in and about Varied Neoplasms

Neoplasm	No. examined	No. with mast cells		Mast cell density*	
		About neoplasm	In neoplasm	About neoplasm	In neoplasm
Transplanted ovarian tumors:					
Luteomas, strain IX	102	52	52	7–19	5–80
Granulosa cell tumors, strain I	9	0	0		
Granulosa cell tumors, strain IV	12	10	0	7–15	
Granulosa cell tumors, strain V	7	5	1	7–10	18
Mixed tumors, strain XI	9	5	0	10–14	
Choriocarcinoma-like line of strain IV	23	14	0	7–18	
X-ray-induced ovarian tumors:					
Tubular adenoma	14	6	5	5	7
Granulosa cell tumor	7	0	0		
Luteoma	7	0	2	3	
Carcinomas:					
Spontaneous	7	2	0	7–9	
Transplanted	9	0	0		
Adenomas, pulmonary, spontaneous	15	0	0		
Sarcomas:					
Miscellaneous, spontaneous	6	0	0		
Miscellaneous, induced	2	0	1		8
Thymic sarcomas	4	3	1	7–10	4
Endotheliomas, transplanted	3	3	1	10–50	12
Leukemias, miscellaneous	44	2	0	5–9	

* The mast cell density is expressed by the number of mast cells in an 0.3 mm.^{2} field of the section. The luteomas[9] and some transmitted granulosa cell tumors[10] have been described elsewhere. Description of the other granulosa cell tumors and of the choriocarcinoma-like growths will follow.[11]

The choriocarcinoma strain had, in the first few passages, cells of the two types indicative of this neoplasm. In the sub-passages these were replaced by endothelium-like cells which caused massive and occasionally exsanguinating hematomas. It seems probable that this is an unusual hemangio-endothelioma.[11]

All x-ray-induced ovarian tumors listed had extended into the periovarian adipose tissue. Of 13 additional tubular adenomas that were limited by the ovarian capsule, only one had mast cells present in the hilar region. In these tumors the mast cells were most numerous about ceroid cell masses and about tubules derived from the germinal epithelium. Of 5 additional granulosa cell tumors that were limited by the capsule, none had mast cells; of 4 additional luteomas, one had mast cells.

Table III warrants no generalization and gives no clue as to why mast cells accumulate in and about some tumors and not about others.

Characteristics of Mast Cell Infiltration in Neoplasms

The distribution of mast cells in luteomas was, in general, similar to that in splenic ER tumors (Figs. 10 and 12), but in luteomas the accumulation of mast cells in the connective tissue was more conspicuous. The mast cells in luteomas were larger (about 18 μ in diameter) than in splenic tumors (about 11 μ in diameter) and, as in ER tumors, they sometimes were seen in capillaries (Fig. 12). As in ER splenic tumors,

the mast cells were in direct contact with the neoplastic cells. An occasional luteoma cell adjacent to mast cells stained metachromatically (Figs. 11 and 12), as did the granules of the mast cells; however, this material showing metachromasia was diffusely distributed in the cytoplasm of the luteoma cells.

Since most tumors studied were in the subcutaneous tissue, it is noteworthy that the number of mast cells in the subcutaneous tissue of the stock of mice studied ranged between two to five in an area 0.3 mm.^{2}, and that the mast cells were concentrated in the adventitia of vessels, in the peri-acinous areas of the mammary gland, and around hair follicles.

In transplanted tumors the mast cell increment was localized in areas where mast cells normally are found. In tumors extending into the subcutaneous fatty tissue, mast cells were especially abundant in connective tissue strands that separated two tumor nodules. The increment of mast cells occurred in foci and was irregularly spotty in distribution. The mast cells in tumors were slightly larger than in the normal skin. The density of granules and their affinity for dyes varied greatly in both. A substance staining metachromatically was not noted outside the mast cells.[12]

Discussion

Character of Mast Cells in the Tumors Studied

Are mast cells neoplastic or hyperplastic? There is no doubt about the neoplastic character of epithelium-like reticulum cells since they multiply progressively by mitotic division with no apparent restraint. The mast cells infiltrating this growth do not appear to be neoplastic. Masses of ER cells can exist without mast cells but the presence of the latter is largely dependent on ER cells. Division forms of mast cells are not seen in small ER nodules. Mast cells are more numerous about strands of stromal connective tissue of the tumor than amidst tumor cells. Furthermore, mast cells are equally abundant in certain luteomas and occur occasionally in tumors of other types.

The known neoplasms of mast cells are monomorphous with admixture of a few other cells of the type encountered in most neoplasms.[1,8,13,14]

Immature mast cells have been well described by Oliver, Bloom, and Mangieri[14] in mastocytomas of dogs. These resemble adult mast cells but have fewer and smaller granules. The ER cells, unlike these mast cell precursors, are free from granules as viewed in the phase microscope and in Giemsa-stained preparations; transitional forms between ER cells and mast cells were never seen.

The data indicate that some tumors stimulate mast cell proliferation or attract mast cells. It is highly probable that this is brought about by some substance present in the tumor or by a metabolite of the tumor cell. There is no known common denominator of neoplasms which contain mast cells. The regular association of ER tumors and luteomas with mast cells suggests experimental studies on the induction of mast cell accumulations by means of subcutaneous injections of cell-free extracts of these tumors.

One exceptional case is noteworthy. In mouse Akh 152, the spleen and bone marrow contained numerous dense foci of mast cells which were also concentrated in the periphery of ER tumors. These mast cell foci resembled those interpreted by Deringer and Dunn[8] as neoplastic. If this interpretation is correct, this animal had two neoplasms which could have arisen independently, or the mast cell hyperplasia in ER tumors changed into neoplasia.

Character and Transplantation Pattern of ER Cells

These epithelium-like splenic tumors originally were described as probably reticular.[7] However, this is uncertain. In the first place, they are unlike the usual reticulum cell sarcomas described in man and animals. These neoplastic cells resemble epithelial cells, notably liver cells, and there is a remote possibility that they are heterotopic epithelial cells.

In a study by LeCompte[15] of tumors of the carotid body, Figure 10 illustrates a tumor with an adenoma-like pattern in which the chief cells have a remarkable epithelium-like arrangement. Some illustrations in that article (Figs. 4, 5, 8, and 10) present a picture indistinguishable from that of our splenic tumors (Figs. 1, 5, and 6). It is conceivable that an organ, such as the spleen, concerned with regulation of blood volume, contains cells which function as do those of the carotid body, but to our knowledge such cells have not been described in the spleen. Further studies are desirable, including the use of tissue cultures and histochemical procedures, to establish beyond doubt the character of the ER cells.

The ER tumors are known only in Ak mice and their hybrids. The rarity of their occurrence (a few in a thousand) is noteworthy because this strain is highly inbred. Accordingly, a high percentage of ER tumors would be expected, or none at all. The difficulties of transplantation in the homologous highly inbred strains are likewise noteworthy.

Histogenesis of Mast Cells

The shape of mast cells and tumor cells, and the appearance of their nuclei and cytoplasms are entirely different and there are no transitional forms between the two. Therefore, the possibility of origin of mast cells from tumor cells can be excluded. Attracted by substances of the tumor cells, the mast cells either migrate into the tumor, or they proliferate in the tumor.

The highly controversial subject of the origin of mast cells has been ably reviewed by Michels.[1] Some workers derive mast cells from "reticulum" cells; others, from histiocytes, "resting" wandering cells, endothelium, "hemohistoblasts," fibroblasts, lymphocytes, and plasma cells.[1] The possibility of origin of mast cells in the bone marrow can be excluded since this organ is almost free from mast cells in mice bearing ER tumors. Our material likewise failed to disclose any evidence of migration of mast cells from blood vessels into the tumor or surrounding connective tissue.

The arguments and observations cited by Michels[1] apply also to the origin of mast cells in luteomas and other tumors. Division figures are scanty and the relation to connective tissue is evident in all. In general, it seems that mast cells increase in connective tissue about the tumor, and later become incorporated in it and continue to proliferate or accumulate in the connective tissue stroma in and about the tumor.

In both ER splenic tumors and luteomas there is a close relationship between tumor cells and mast cells in that the mast cells surround the tumor cells by cytoplasmic extensions. In luteomas a metachromatic staining is noted in a few cells adjacent to mast cells. This is not due to imbibition of dissolved granules or to insufficient dehydration of the sections (false metachromatic staining of Sylvén[12]) because the color is within the boundaries of the luteoma cells.

There is much discussion in the older literature concerning the motility of mast cells,[1] but Paff, Bloom, and Reilly[16] recently have demonstrated a slow ameboid motion of mast cells by means of motion pictures. Earlier, Lehner[17] had observed in the mesentery and other tissues of the frog, retraction of cytoplasmic processes and movement of granules in mast cells.

The relationship between mast cells of tissue and blood has not been established definitely, although most observers believe that they are unrelated.[1,18] In a transplantable mast cell leukemia of mice[19] the bone marrow was a frequent site of mast cell proliferation and, unlike cells of mastocytomas, the neoplastic mast-myelocytes behaved as other leu-

kemic leukocytes. The bone marrow is almost entirely devoid of tissue mast cells in both normal mice and in mice bearing ER tumors rich in mast cells. Both shape and size are variable, as exemplified by the behavior of monocytes which are spherical in the blood, assuming microglia-like forms in tissues and large epithelioid forms in reactive processes. Accordingly, shape and size are not trustworthy characteristics to distinguish mast cells of tissue from those of blood. Although mast cells of tissue and blood appear different morphologically and histogenetically, their metachromatic cytoplasmic granules appear similar and functionally these two types may be related. The term "heparinocyte" has been suggested[3] for mast cells of the blood, but this term should include mast cells of tissue as well; or, according to Jorpes,[3] should preferably be given only to mast cells of tissue.

Very recently, Barnard[20] mentioned coincident hyperheparinemia, basophilic myelocytosis, and estrapenia in 3 cases of purpura. He postulated that hemal mast cells of marrow origin are responsible for heparin production and that the heparin level regulates thromboplastin production by the bone marrow. Such an equilibrating mechanism would satisfactorily explain failure of hemorrhagic tendencies in animals bearing tumors rich in mast cells, although it is equally conceivable that the mast cell proliferation is secondary to a hypothetic thromboplastic substance elaborated by tumors.

The Function of Mast Cells

Opinions on the supposed function of mast cells have been well reviewed by Michels[1] and only a few will be mentioned here. The recent discovery of the chemical composition of mast cell granules pushed aside the earlier, more or less speculative assumptions on the function of mast cells and linked them with two seemingly unrelated processes: (a) production of the ground substance of connective tissue, and (b) the blood clotting mechanism. There is suggestive evidence for both. These two functions appear unrelated unless it is postulated that the polyester sulfuric acid contributed by mast cells to the connective tissue helps to maintain the semi-fluidity or gel-like character of the connective tissue, thus facilitating migration of leukocytes and vital processes.

Staemmler[21] is the strongest proponent of the view that mast cells are related to the intercellular connective tissue substance. He noted a direct correlation between the amount of loose connective tissue and the mast cell content of tumors, in line with his thesis that mast cells aid the formation of connective tissue by contributing "mucin" to it. The trans-

plantable ER tumors in which we noted an abundance of mast cells may be related to reticulum cells, but other more typical reticulum cell tumors did not contain mast cells. The transplantable luteomas characteristically rich in mast cells were relatively poor in connective tissue.

In the opinion of Sylvén,[12] mast cells give to the collagenous fibers a "free chromotrope substance," a polyester of sulfuric acid, during their breakdown, brought about by a proteolytic enzyme derived from the tumor. Sylvén found a mast cell increment and free chromotrope substance only in very rapidly growing parts of mesenchymal tumors. The mast cell increment in our ovarian tumors was not related to the state of differentiation or rapidity of tumor growth, and, as already stated, the luteomas rich in mast cells were unusually poor in connective tissue stroma.

It is noteworthy that a hemorrhagic tendency was not apparent in mastocytoma-bearing animals or in mice with tumors rich in mast cells. Heparin has been shown to inhibit cell growth (Zakrzewski[22] and Fischer[23]), and the opinion that mast cells inhibit tumor growth has been expressed by numerous investigators. Fromme[24] and others believed that mast cells aid the formation of connective tissue barriers about tumors and neutralize "toxins" of cancer cells.

The relation between carcinogens and mast cell increment has been the subject of numerous studies. Fabris[25] exposed mice to an atmosphere of finely pulverized tar. These animals developed nodules and circumscribed agglomerations of mast cells with plasma cells in the papillary layer of the skin, notably in the cheeks, shoulders, feet, ears, and inguinal region. Franco and Affonso[26] observed an immense increment of mast cells in the skin after 67 applications of tar. Schreus[27] painted mice with neutral tar oil and noted a nodule in subcutaneous tissue composed entirely of mast cells. Twort and Twort,[28] using oil and tar, obtained mast cell tumors in the skin near the dermis, one of which was invasive. Cramer and Simpson[2] observed a particularly marked mast cell increment about the benign epithelial hyperplasia occurring following methylcholanthrene treatment.

Thus it is observed that certain agents, including some carcinogens and some tumor cells, stimulate mast cells; the ability of certain tumor cells to do so seems to be a characteristic property of the tumor cells, but the agent directly responsible for mast cell stimulation is unknown as is the meaning of mast cells in tumors.

That the function of mast cells is more complex than mere elaboration of heparin is suggested by the recent findings of acid[29] and alkaline

phosphatases and cytochrome oxidase[30] in mast cell granules, and also sudanophile lipoids.[29,31]

If mast cells produce heparin, the question remains to be answered why some tumors contain them in abundance and others contain none. It is conceivable that metabolites or autolytic products of tumor cells promote blood clotting to a varying extent and that mast cells counteract these blood clotting substances.

Further chemical and hematologic studies are desirable to learn about the functions of mast cells. The transplantable tumors rich in mast cells may serve that purpose.

Summary

A benign transplantable splenic tumor occurs in the Ak strain of mice, with neoplastic cells resembling epithelium. These cells are probably related to reticulum cells.

These neoplasms, both spontaneous and transplanted, are characteristically rich in mast cells. Histogenetically, the mast cells are not related to the neoplastic cells. They appear to be tissue cells not derived from the bone marrow or other hemopoietic organs.

Mast cells are characteristically present in certain neoplasms and are absent in others. Among the former are luteomas, both spontaneous and transplanted; among the latter are granulosa cell tumors, pulmonary adenomas, breast cancers, and malignant lymphomas.

The theory is favored that a dynamic equilibrium exists between blood clotting (thromboplastic) substances and the fluidity-maintaining substances (heparin). This gives a meaning to mast cell proliferation and explains why animals bearing tumors rich in mast cells do not exhibit hemorrhagic tendencies.

REFERENCES

1. Michels, N. A. The Mast Cells. In: Downey, H. (ed.) Handbook of Hematology. Paul B. Hoeber, Inc., New York, 1938, **1**, 231–372.

2. Cramer, W., and Simpson, W. L. Mast cells in experimental skin carcinogenesis. *Cancer Research,* 1944, **4**, 601–616.

3. Jorpes, J. E. Heparin in the Treatment of Thrombosis. Oxford University Press, London, 1946, ed. 2, 260 pp.

4. Holmgren, H., and Wilander, O. Beitrag zur Kenntniss der Chemie und Funktion der Ehrlichschen Mastzellen. *Ztschr. f. mikr.-anat. Forsch.,* 1937, **42**, 242–278.

5. Jorpes, E., Holmgren, H., and Wilander, O. Über das Vorkommen von Heparin in den Gefässwänden und in den Augen. Ein Beitrag zur Physiologie der Ehrlichschen Mastzellen. *Ztschr. f. mikr.-anat. Forsch.,* 1937, **42**, 279–301.

6. Allen, J. G., Sanderson, M., Milham, M., Kirschon, A., and Jacobson, L. O. Heparinemia (?). An anticoagulant in the blood of dogs with hemorrhagic

tendency after total body exposure to roentgen rays. *J. Exper. Med.*, 1948, **87**, 71–86.

7. Furth, J., and Krumdieck, N. Splenic reticulum cell tumors in mice. *Am. J. Path.*, 1940, **16**, 449–456.

8. Deringer, M. K., and Dunn, T. B. Mast-cell neoplasia in mice. *J. Nat. Cancer Inst.*, 1946–47, **7**, 289–298.

9. Furth, J., and Sobel, H. Transplantable luteoma in mice and associated secondary changes. *Cancer Research*, 1947, **7**, 246–262.

10. Furth, J. Transplantability of induced granulosa cell tumors and of luteoma in mice. Secondary effects of these growths. *Proc. Soc. Exper. Biol. & Med.*, 1946, **61**, 212–214.

11. Bali, T., and Furth, J. Morphological characteristics of x-ray induced transplantable ovarian neoplasms. (In preparation.)

12. Sylvén, B. Ester sulphuric acids of high molecular weight and mast cells in mesenchymal tumors. *Acta radiol.*, 1945, suppl. 59, pp. 1–99.

13. Bloom, F. Spontaneous solitary and multiple mast cell tumors ("mastocytoma") in dogs. *Arch. Path.*, 1942, **33**, 661–676.

14. Oliver, J., Bloom, F., and Mangieri, C. On the origin of heparin. An examination of the heparin content and the specific cytoplasmic particles of neoplastic mast cells. *J. Exper. Med.*, 1947, **86**, 107–116.

15. LeCompte, P. M. Tumors of the carotid body. *Am. J. Path.*, 1948, **24**, 305–321.

16. Paff, G. H., Bloom, F., and Reilly, C. The morphology and behavior of neoplastic mast cells cultivated *in vitro*. *J. Exper. Med.*, 1947, **86**, 117–124.

17. Lehner, J. Das Mastzellen-problem und die Metachromasie-Frage. *Ergebn. d. Anat. u. Entwcklngsgesch.*, 1924, **25**, 67–184. (Cited by Michels.[1])

18. Doan, C. A., and Reinhart, H. L. The basophil granulocyte, basophilcytosis, and myeloid leukemia, basophil and "mixed granule" types; an experimental, clinical and pathological study, with the report of a new syndrome. *Am. J. Clin. Path.*, 1941, **11**, 1–39.

19. Furth, J. Transmission of myeloid leukemia of mice; its relation to myeloma. *J. Exper. Med.*, 1935, **61**, 423–445.

20. Barnard, R. D. Similarity to heparin of the clotting inhibitor in acute leucemia and the significance of hyperheparinemia in estrapenic cholinergic states. *Science*, 1948, **107**, 571–572.

21. Staemmler, M. Untersuchung über Vorkommen und Bedeutung der histiogenen Mastzellen im menschlichen Körper unter normalen und pathologischen Verhältniss :n. *Frankfurt. Ztschr. f. Path.*, 1921, **25**, 391–435.

22. Zakrzewski, Z. Untersuchungen über den Einfluss von Heparin auf das Wachstum der Sarkomzellen in vitro. *Ztschr. f. Krebsforsch.*, 1932, **36**, 513–528. (Cited by Cramer and Simpson.[2])

23. Fischer, A. Über die Wirkung der Heparins auf das Wachstum von Gewebezellen in vitro. *Protoplasma*, 1936, **26**, 344–350. (Cited by Cramer and Simpson.[2])

24. Fromme, F. Demonstration über das Verhalten der Mastzellen beim Karzinom. *Zentralbl. f. Gynäk.*, 1906, **30**, 1146. (Cited by Michels.[1])

25. Fabris, A. "Mastocitomi" cutanei da catrame. *Pathologica*, 1927, **19**, 157–166. (Cited by Michels.[1])

26. Franco, E. E., and Affonso, C. Sulla proderzione del cancro e sul comportamento delle mastzellen nei topi trattati col catrame. *Pathologica*, 1924, **16**, 445–450. (Cited by Fabris.[25])

27. Schreus, H. T. Ueber einen Mastzellen-Tumor bei der weissen Maus nach Teerpinselung. *Dermat. Ztschr.*, 1923–24, **40**, 9–14. (Cited by Cramer and Simpson.[2])

28. Twort, C. C., and Twort, J. M. Classification of four thousand experimental oil and tar skin tumours of mice. *Lancet*, 1930, **1**, 1331–1335. (Cited by Deringer and Dunn.[8])

29. Wislocki, G. B., Bunting, H., and Dempsey, E. W. Further observations on the chemical cytology of megakaryocytes and other cells of hemopoietic tissues. *Anat. Rec.*, 1947, **98**, 527–537.

30. Noback, C. R., and Montagna, W. Some histochemical aspects of the mast cell with special reference to alkaline phosphatase and cytochrome oxidase. *Anat. Rec.*, 1946, **96**, 279–287.

31. Sehrt, E. Die histologische Darstellung der Lipoide der weissen Blutzellen (neutro- und eosinophile Leukozyten, Mastzellen, Uebergangszellen und Mononukleäre) und die Beziehung dieser Lipoide zur Oxydasereaktion. *München. med. Wchnschr.*, 1927, **74**, 139–141.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 93

FIG. 1. Spontaneous ER tumor in the spleen. Among the nests of tumor cells, erythropoietic and granulopoietic foci are seen. The mast cells are unstained. Hematoxylin and eosin stain. × 450.

FIG. 2. Mast cells in a spontaneous splenic ER tumor, showing variability in their shape and size. Giemsa's stain. × 100.

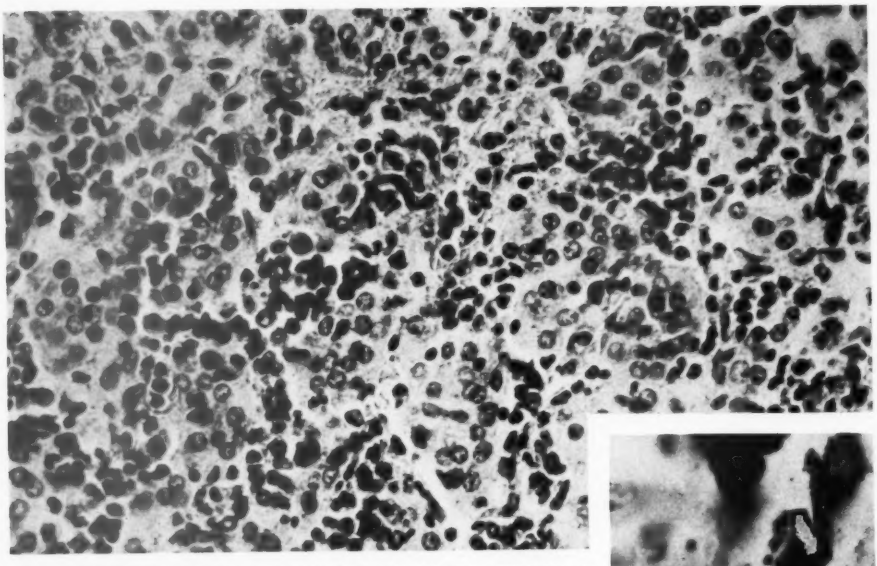
FIG. 3. An area from Figure 2, showing mast cells and a binucleated cell in the center of the field. Giemsa's stain. × 950.

FIG. 4. A chain of mast cells in the bone marrow of the only case (Akh 152) in which mast cells were abundant in this organ. Giemsa's stain. × 950.

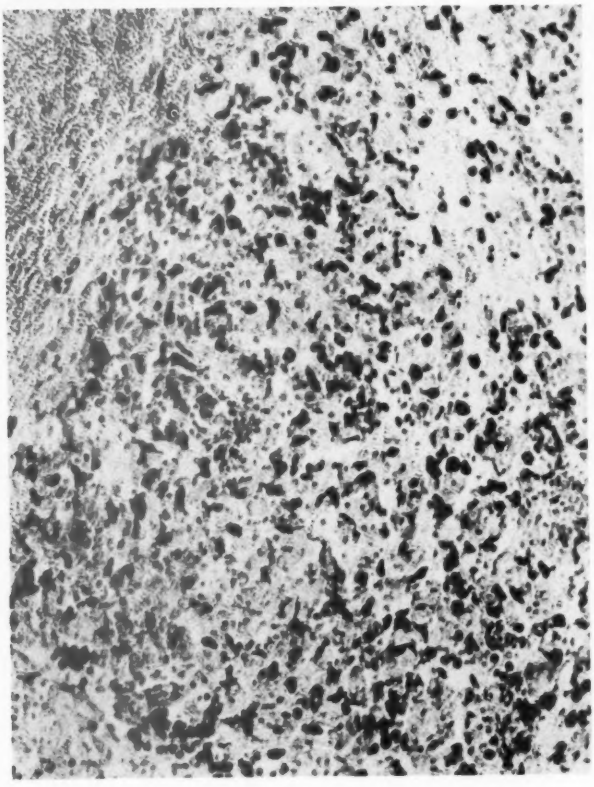
1

2

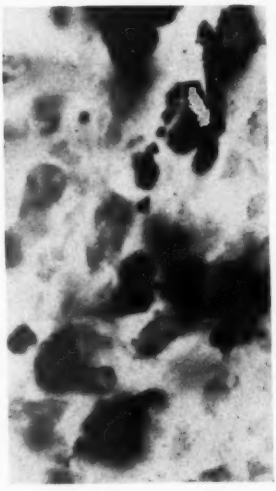
1



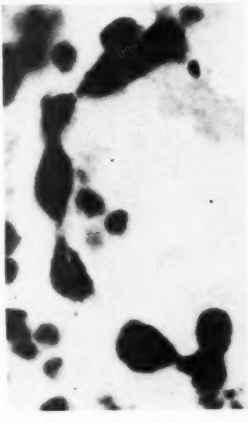
2



3



4



Bali and Furth

Splenic Tumor Rich in Mast Cells

PLATE 94

FIG. 5. Spontaneous splenic ER tumor, the only one that was devoid of mast cells. Hematoxylin and eosin stain. × 570.

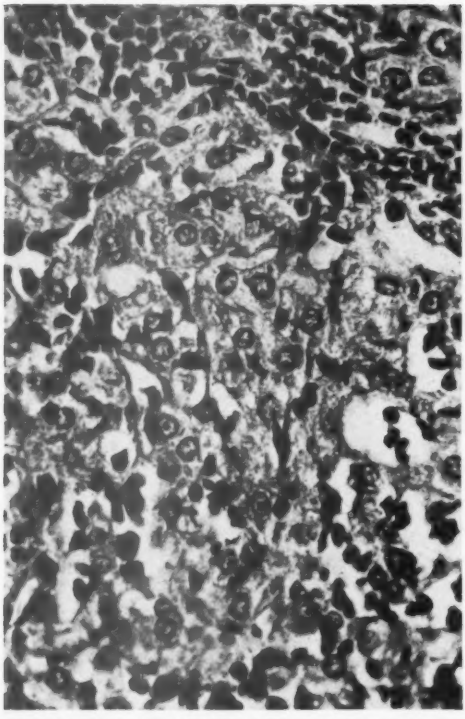
FIG. 6. A subcutaneously transplanted ER tumor with neoplastic cells in trabecular arrangement, with wide capillary spaces among them. This picture resembles closely that of the carotid body tumor of Le Compte.[15] Hematoxylin and eosin stain. × 850.

FIG. 7. General appearance of a transplanted ER tumor. Hematoxylin and eosin stain. × 100.

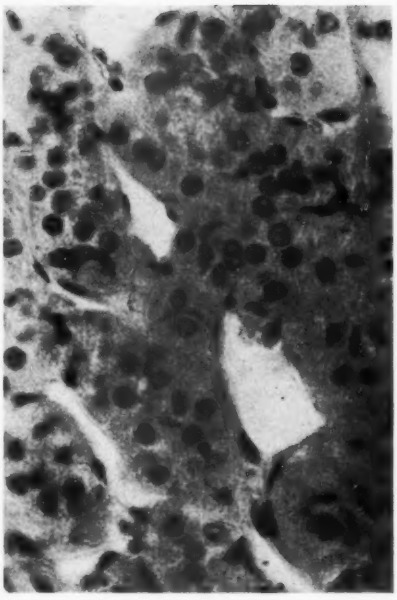
FIG. 8. Two mast cells from a spontaneous splenic ER tumor that appear to be connected by cytoplasmic processes, a picture frequently encountered. Giemsa's stain. × 950.

FIG. 9. A subcutaneously transplanted ER tumor with a mitotic figure in the center. The epithelium-like appearance of the tumor cells is particularly well shown. Hematoxylin and eosin stain. × 950.

5

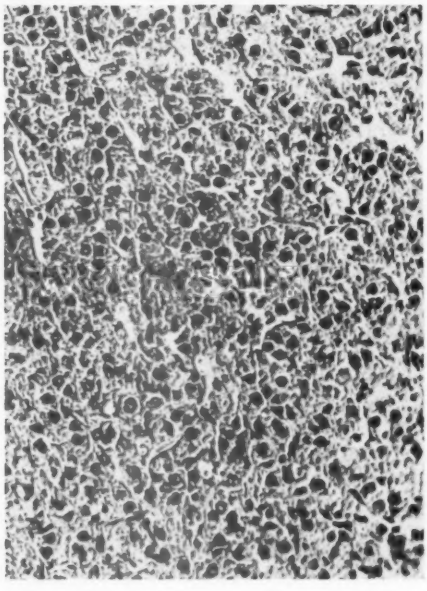


6

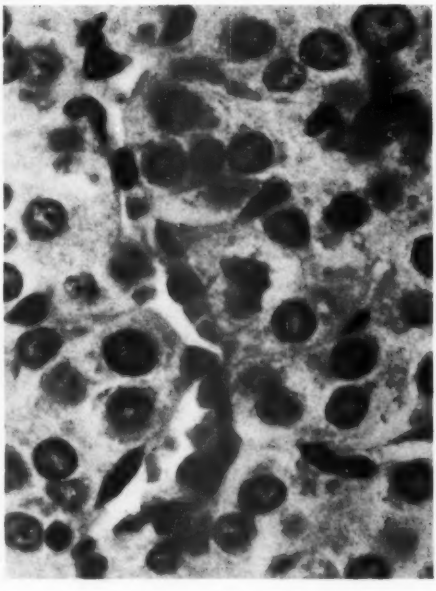


8

7



9



Bali and Furth

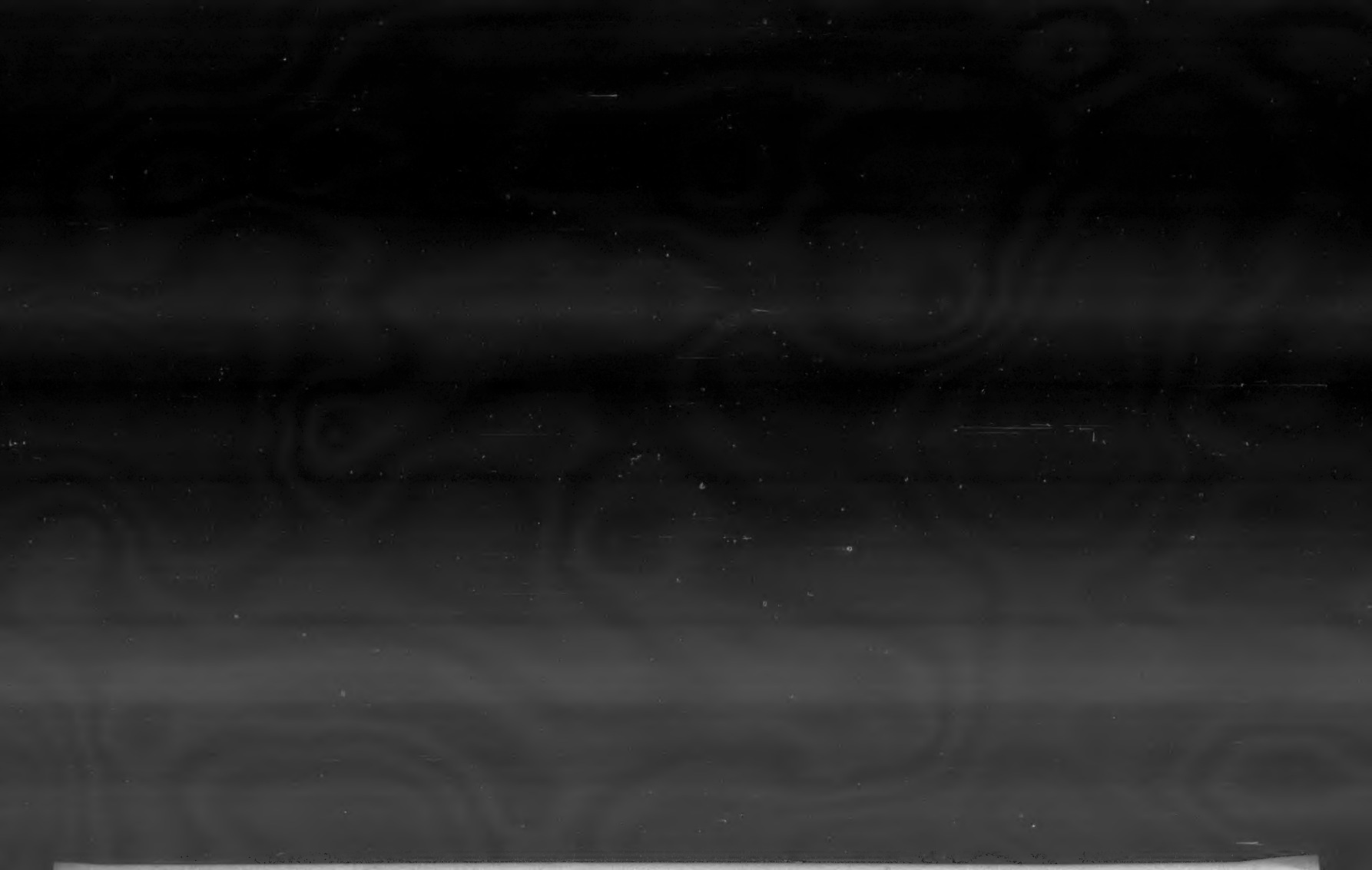
Splenic Tumor Rich in Mast Cells

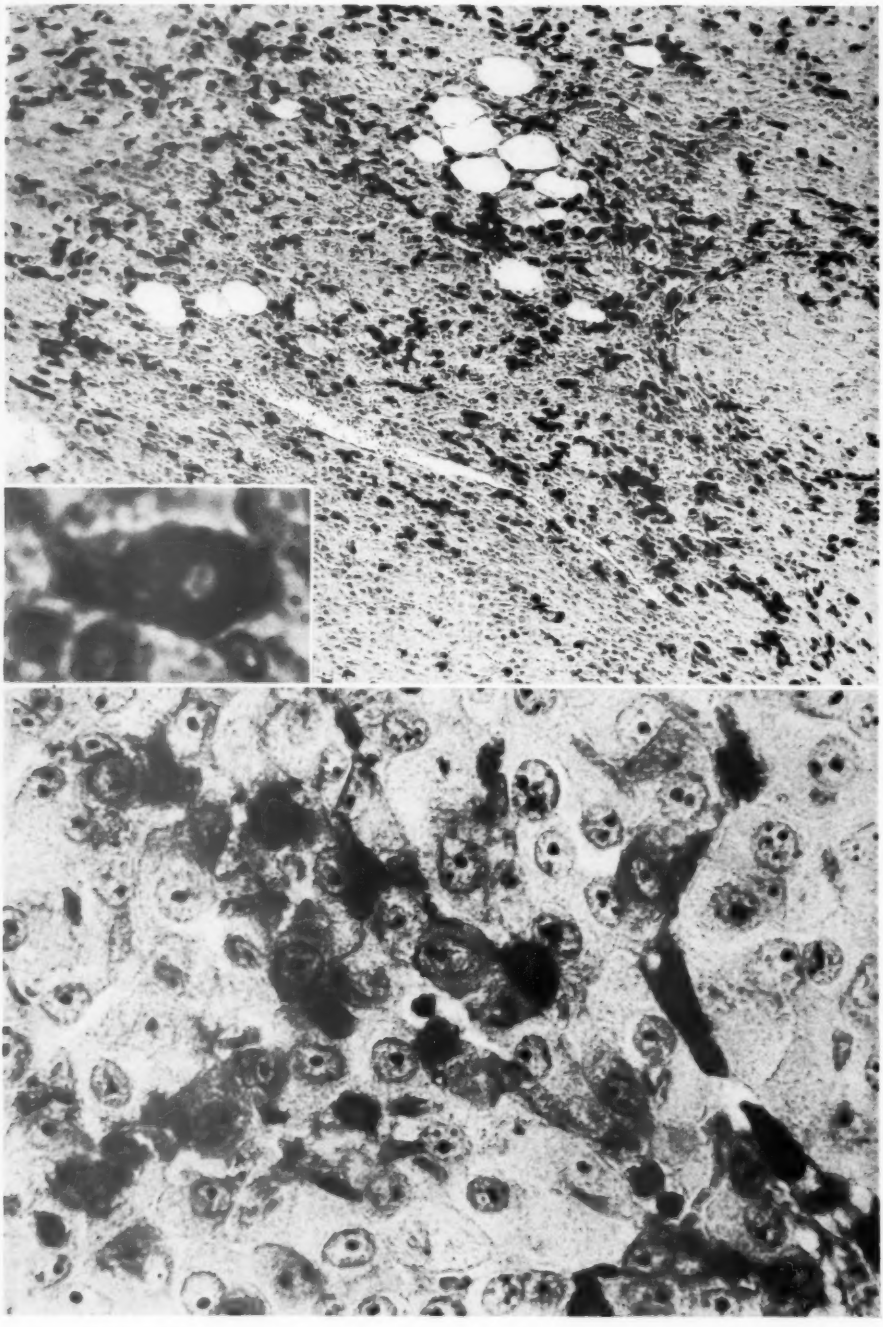
PLATE 95

FIG. 10. Transplanted luteoma (strain IX) in subcutaneous fatty tissue. Mast cells are absent in the central parts of an apparently young tumor nodule close to the right margin of the field, but are abundant in the peripheral parts of the nodule and in other, presumably older, parts of the tumor. Giemsa's stain. × 200.

FIG. 11. A sharply demarcated, unstained vacuole in a mast cell in a luteoma. Giemsa's stain. × 970.

FIG. 12. Luteoma cells, metachromatically stained, adjacent to mast cells. The two mast cells in the right lower field are in a capillary. Giemsa's stain. × 950.





Bali and Furth Splenic Tumor Rich in Mast Cells

SEBACEOUS GLANDS IN NORMAL AND NEOPLASTIC PAROTID GLANDS

Possible Significance of Sebaceous Glands in Respect to the Origin of Tumors of the Salivary Glands *

Luis Meza-Chávez, M.D.†

(From the Department of Pathology of the University of Michigan, Ann Arbor, Mich.)

The presence of structures resembling sebaceous glands in two parotid glands that contained adenomas has been mentioned in another publication.[1] In those cases it was observed that such structures arose from the intralobular ducts. In one instance, sebaceous glands were seen in an area of tumor formation as well as in the normal parotid tissue. However, in the other they were present only in the normal portion. The glandular structures were in all respects similar to the sebaceous glands found in areas where they are not associated with hairs. Their occurrence in parotid glands is not mentioned in any of the standard textbooks of histology. Search of the literature revealed two reports in which similar findings have been recorded. Hamperl[2] observed holocrine fat secretion (sebaceous-like transformation) in the excretory system in four submaxillary glands of 4 of 85 persons from whom he studied the uvular, sublingual, and submaxillary glands, and in one parotid gland. Hamperl stated that he found sebaceous-gland-like transformation in small intralobular ducts ending blindly. The ages of the persons in whom this transformation was found in the submaxillary glands were 6½, 9, 20, and 36 years, respectively, and the person whose parotid gland showed the sebaceous-gland-like transformation was 6 years old.

The only other report of sebaceous glands in the parotid gland is that of Hartz.[3] The individual in whom they were found was a 34-year-old woman who had a recurrent mixed tumor of the left parotid gland. The tumor was removed with the surrounding glandular tissue. The microscopic study of the specimen showed a typical "mixed tumor" and some normal parotid gland in which Hartz found small sebaceous glands originating from the striated or intercalated ducts. He had the impression that the sebaceous glands developed from short branches that ended blindly. This author pointed out that it was not known whether such structures "were present before the tumor and the first operation or whether those abnormal circumstances were the stimulus for the peculiar differentiation of the ductal epithelium."

Because of these findings it was thought that further study was neces-

* Received for publication, July 20, 1948.

† Hubert E. Rogers' Fellow in Pathology.

sary to determine (1) whether sebaceous glands are present in normal parotid glands, (2) whether they are present in parotid glands which are the site of a neoplastic process, and (3) whether or not the occurrence of such structures in the salivary glands could have any bearing on the explanation of the tumors occurring in these salivary glands. With these questions in mind, it was decided to study normal parotid glands, and those in which tumors had occurred.

Material and Methods

The material for this study was divided into two groups: (1) tumors of the parotid gland around which there was normal salivary gland tissue, and (2) normal parotid glands obtained from autopsy material.

The first group consisted of prepared sections from 249 tumors of the parotid gland of which only 133 showed some normal salivary gland tissue about the tumor. These latter slides were used for the present study. With the exception of one case from Memorial Hospital, New York City, all came from the Department of Pathology of the University of Michigan. They represented the tumors of the parotid gland diagnosed during the preceding 15 years, which will be considered together and will be referred to as group 1 throughout this communication (Table I).

Table I

The Material of Group 1 (Tumor Cases) with Percentage of Those That Had Sebaceous Glands

Number of tumors	249
Number of tumors with normal parotid gland attached	133
Number of cases with sebaceous glands	33
Percentage of cases with sebaceous glands of those with parotid gland attached	24.8

The amount of normal parotid tissue present varied considerably, but as a rule it was not very abundant. In order to be included in this study, it was required that at least one of the sections show 25 mm.^{2} of normal salivary gland. All of the sections from this group were stained with hematoxylin and eosin.

The second group was composed of 100 normal parotid glands obtained from 51 persons who died from various causes, including accidents and violence. These were unselected individuals on whom autopsies were performed at the University Hospital. Their ages ranged from 7 months to 76 years. Thirty-three were males. Both parotid glands were available for study from 49 individuals and from each of 2, only one gland could be obtained. The amount of parotid tissue removed from each person was about two-thirds of each gland in most cases. Formalin was used to

fix 80 of the 100 parotid glands and the remaining 20 were fixed in absolute alcohol. An average of two blocks of tissue were imbedded in paraffin from each gland and five sections obtained from each block were stained with hematoxylin and eosin. Hortega's silver carbonate nuclear stain on frozen sections[4] was done in every case in which sebaceous glands were found, provided the material had been fixed in formalin. This was followed by scharlach R staining for fat in every section which showed sebaceous glands in the silver impregnation. Mayer's mucicarmine stain was used on material fixed either in formalin or in alcohol, and Best's carmine stain when the sections showed sebaceous glands and the material had been fixed in absolute alcohol.

Microscopic Findings

The microscopic findings were similar in both groups. The parotid glands presented the normal tubulo-acinar structure and they varied only in degree of atrophy, as recorded in Table II. Most of the ducts were normal, but in areas changes had taken place in some branches of the striated ducts or more frequently in the intercalated ducts. These branches or ducts usually were short and had blind ends, instead of branching normally into intercalated ducts or ending in glandular acini. The blind ducts were lined by low-cuboidal epithelium that progressively changed into the stratified squamous type. This change was sometimes very abrupt.

Some of these ducts were curved, forming small hooks which often ended in saccular or alveolar epithelial structures which varied in size from 50 to 350 μ. They were spherical, ovoid, or elongated, and were composed of a well defined basal membrane over which lay a row of cuboidal or flattened epithelial cells (Figs. 1 to 8). Sometimes these cells formed small groups composed of several layers situated about the periphery of the alveolus or toward the lumen of the duct (Figs. 2, 3, 4, and 8). Their nuclei were round or oval and larger than those of the cells of the parotid acini or of secretory or intercalated ducts (Figs. 1, 2, 5, and 6). They usually showed a small nucleolus, although occasionally two nucleoli could be seen. The cells increased in size toward the center of the alveolar mass, where they were spherical, polyhedral, or irregular in shape. Their cytoplasm was vacuolated, the vacuoles being larger in the cells more centrally situated or close to the opening of the alveolus into the lumen of the duct (Figs. 1 to 8). Some of the nuclei of these cells were similar to those of the peripheral cells, but others were irregular and pyknotic. In some cells no nuclei could be seen. In many cases the

TABLE II

Data on the Cases in Which the Parotid Glands Were the Site of Tumors and in Which Sebaceous Glands Also Were Found

No.	Sex	Age	Side	Diagnosis of the tumor	Amount of parotid gland available	State of the parotid	Sebaceous glands
1	F	23	?	Mixed tumor	Small piece	Normal	+
2	F	33	?	Mixed tumor	Small piece	Slight atrophy	++
3	?	?	?	Muco-epidermoid tumor	Very small piece	Slight atrophy	+
4	F	60	?	Adenoma	Very small piece	Moderate atrophy	+
5	F	68	?	Mixed tumor	Very small piece	Moderate atrophy	+
6	F	52	Left	Muco-epidermoid tumor	Small piece	Normal	+
7	M	35	?	Mixed tumor	Small piece	Normal	+
8	M	31	Left	Mixed tumor	A piece	Slight atrophy	+
9	M	42	Left	Mixed tumor	Small piece	Moderate atrophy	+
10	M	21	Right	Mixed tumor	Small piece	Slight atrophy	+
11	M	24	Right	Mixed tumor	Very small piece	Slight atrophy	+
12*	M	50	Left	Adenoma	Small piece	Slight atrophy	++++++
13	F	19	Left	Mixed tumor	Small piece	Slight atrophy	+
14	F	31	Left	Mixed tumor	A piece	Slight atrophy	++
15	F	25	Right	Carcinoma	A piece	Moderate atrophy	++
16	M	5	Left	Carcinoma	Small piece	Slight atrophy	++
17	M	34	Left	Muco-epidermoid tumor	Small piece	Slight atrophy	++++++
18	M	21	Left	Muco-epidermoid tumor	A piece	Slight atrophy	+
19	F	38	Left	Muco-epidermoid tumor	Small piece	Slight atrophy	+
20	M	36	Left	Carcinoma	Small piece	Moderate atrophy	+
21	M	34	Right	Muco-epidermoid tumor	Small piece	Slight atrophy	+
22	M	57	Right	Mixed tumor	A piece	Moderate atrophy	+
23	M	44	Right	Muco-epidermoid tumor	A piece	Moderate atrophy	+
24	F	17	Left	Carcinoma	A piece	Moderate atrophy	+
25	F	34	Left	Carcinoma	A piece	Slight atrophy	+
26	M	28	Right	Mixed tumor	Small piece	Normal	+
27	M	77	Left	Mixed tumor	Small piece	Atrophy	+
28	M	46	Right	Mixed tumor	Small piece	Moderate atrophy	+
29	F	40	Right	Mixed tumor	A piece	Moderate atrophy	++++++
30	F	47	Right	Mixed tumor	Very small piece	Atrophy	+
31	F	37	Left	Muco-epidermoid tumor	Very small piece	Slight atrophy	+
32	M	57	Right	Carcinoma	Small piece	Moderate atrophy	+
33	F	51	Left	Mixed tumor	A piece	Slight atrophy	+

* Case from Memorial Hospital, New York City.

large cells with shrunken nuclei were separating into the lumen of the duct, forming a holocrine secretion (Figs. 1, 4, and 7). Mitotic figures were seen only in the cells of the periphery of the alveoli (Fig. 7). The alveoli usually were single at the end of the blind ducts but sometimes two or three alveoli opened into a duct to form a simple branched gland (Fig. 8). In frozen sections, stained with Hortega's silver carbonate, it was seen that the empty vacuoles, observed in the paraffin sections, were filled with a translucent, refringent substance. When these same sections were stained subsequently with scharlach R, the substance contained in the vacuoles varied from orange to red. Sections from paraffin-imbedded tissue stained with Mayer's mucicarmine and Best's carmine failed to show mucin or glycogen in the vacuoles. The presence of sebaceous-gland-like structures was demonstrated in frozen sections from only 14 of 22 parotid glands which showed them in the paraffin sections and which were saved in formalin, although hundreds of sections were studied from the remaining 8 cases.

Comparative Study of the Findings in Both Groups

From the microscopic findings, it was evident that the epithelial structures found in the parotid glands had morphologic and tinctorial characteristics similar to those of sebaceous glands, particularly of the kind seen in areas where such glands are not associated with hair. These structures will be designated as sebaceous glands in the discussion which follows.

The findings and certain clinical data for the patients in whom the sebaceous glands were found are summarized in Tables II and III, but some of the facts recorded there should be emphasized.

Incidence of Sebaceous Glands. Thirty-three parotid glands of group 1 showed sebaceous glands and 28 in group 2, which represents 24.8 per cent and 28 per cent, respectively. In group 2, sebaceous glands were found in both parotid glands in 11 persons and in one parotid gland only in 6 persons, making a total of 17. In other words, 33 per cent of the persons whose parotid glands were examined at autopsy showed sebaceous glands.

Incidence in Relation to Sex. The 33 parotid glands of group 1 that showed sebaceous glands were from 17 males and 15 females, with one person whose sex was not recorded. In group 2, 12 of those in whom sebaceous glands were found were male and 5 female. This apparent preponderance in the male sex is not significant, because 33 persons of this group were male and only 18 female.

Incidence in Relation to Age. The occurrence of sebaceous glands

TABLE III

Data on the Autopsy Cases in Which the Parotid Glands Showed Sebaceous Glands

No.	Sex	Age	Cause of death	State of the parotid	Sebaceous gland	Remarks
1*	M	70	Bronchial asthma, retroperitoneal hemorrhage	Moderate atrophy	+	Positive fat stain
2	M	67	Myocardial infarction, squamous cell carcinoma of tongue and floor of mouth	Moderate atrophy	++	Positive fat stain
3	M	54	Hypernephroma of left kidney with multiple metastases, diabetes	Slight atrophy	+	No sebaceous gland found in frozen sections
4*	M	76	Carcinoma of stomach, bronchopneumonia	Marked atrophy	+	No sebaceous gland found in frozen sections
5*	F	21	Spongioblastoma polare, acute purulent lobular pneumonia	Slight atrophy	+	Positive fat stain
6*	M	22	Subdural hematoma, multiple fractures	Moderate atrophy	+	No sebaceous gland found in frozen sections
7*	F	49	Carcinoma of the breast, with multiple metastases	Slight atrophy	++	No sebaceous gland found in frozen sections
8	F	64	Third-degree burns, carcinoma of the thyroid	Slight atrophy	+	No sebaceous gland found in frozen sections
9	M	43	Myelosarcomatosis, bronchopneumonia	Slight atrophy	+++	Positive fat stain
10	M	25	Chronic glomerulonephritis, uremia, peptic ulcer	Normal	+	Sebaceous glands negative for glycogen and mucin
11	F	15	Polycythemia, massive infarction of occipital and parietal portion of brain	Slight atrophy	+	Sebaceous glands negative for glycogen and mucin
12	M	13	Medulloblastoma of cerebellum invading meninges and spinal cord	Slight atrophy	+	Positive fat stain
13	F	36	Dicoumarol poisoning	Slight atrophy	+	Positive fat stain
14	M	6	Encephalitis	Normal	++	Positive fat stain
15	M	21	Epidural hemorrhage	Normal	++	Positive fat stain
16	M	19	Bullet wound	Normal	++	Positive fat stain
17*	M	50	Multiple bullet wounds	Normal	+	Positive fat stain

* Cases in which only one parotid gland showed sebaceous glands.

had no relation to age, as can be seen clearly in Table IV, which shows that the youngest person was 5 years old and the oldest 77 years in group 1, and 6 years and 76 years in group 2. The concentration noted from the second to the sixth decade in group 1 probably cannot be considered significant since that is a period in which most salivary gland tumors occur.

TABLE IV

Age Distribution by Decades of Cases with Sebaceous Glands

Age	Tumor cases* (group 1)	Autopsy cases (group 2)
1–10	1 (Youngest 5 years)	1 (Youngest 6 years)
11–20	2	3
21–30	7	4
31–40	10	1
41–50	5	3
51–60	5	1
61–70	1	3
71–80	1 (Oldest 77)	1 (Oldest 76)

* Age not known for 1 case.

Sebaceous Glands and Glandular Atrophy. There was no association between atrophy of the parotid gland and the occurrence of sebaceous glands since they were found in parotid glands showing all degrees of atrophy or no atrophy at all.

Number and Degree of Development of the Sebaceous Glands. The study of the material in relation to frequency and to degree of development showed that sebaceous glands were more numerous in the cases of group 1, in which they also reached a larger size and sometimes presented a branched appearance, while in group 2 they were less numerous, smaller, and only one branched gland was seen (Figs. 14 to 20). The average number of sebaceous glands found in the sections from each case of group 1 was 4.5, while in group 2 it was 2.5. These figures, although significant, do not adequately express the preponderance clearly observed in the study of the material, for it was noticed that there was greater frequency of occurrence of two or more sebaceous glands in one microscopic field in cases from group 1 (Figs. 9 to 13), while in group 2 on only two occasions were two sebaceous glands seen in one field (Fig. 14). This lack of agreement between observation and tabulation can be explained by the fact that the amount of normal parotid gland available for study from each case of group 1 was often one or two small sections, while in cases from group 2 at least 10 sections obtained from two different blocks were studied. In regard to size, the largest sebaceous glands were seen in parotid glands of group 1 in which they reached 350 μ in diameter.

Relation of Sebaceous Glands and Type of Tumor. Sebaceous glands were found in association with 17 mixed tumors, 8 neoplasms of the muco-epidermoid type recently separated from the so-called mixed tumor group,[5] 6 carcinomas, and 2 adenomas. The great frequency with which sebaceous glands were present in parotid glands that were the site of muco-epidermoid tumors should be noticed. This can be brought out better by two facts. First, examples of this type comprised 24 per cent of the tumors associated with sebaceous glands. This figure is very high if one considers that the incidence of the muco-epidermoid tumor in relation to all salivary gland tumors is 5+ per cent.[5] Second, sebaceous glands were found in 8 of 11 cases of muco-epidermoid tumor in which there was normal parotid gland present; or, in other words, they were found in 73 per cent of the parotid glands that were the site of such tumors. The association of sebaceous glands and carcinoma in the same parotid gland also seems to be of interest, because this occurred in 18 per cent of the cases of group 1 in which sebaceous glands were seen. This is a higher incidence than that of carcinoma in relation to mixed tumors, which was estimated to be 14+ per cent in the material studied for this report. This figure comes close to the percentage for malignant tumors of the parotid gland found by Stein and Geschickter,[6] which was 17 per cent, and the 15+ per cent obtained from the cases studied by Benedict and Meigs,[7] who had 30 examples of carcinoma in 203 cases of parotid tumors.

Comments

If it be accepted that the epithelial structures found in parotid glands are truly sebaceous glands, it becomes necessary to explain how these accessory skin structures happen to be present in this location. Their occurrence in the mucosa of the mouth, where they have long been recognized and their presence designated as Fordyce's disease, has been explained as resulting from aberrant buds along the fetal line of closure.[8,9] This explanation cannot be accepted for their occurrence in the parotid and submaxillary glands, because there is no line of closure in those glands and because there is definite proof that these sebaceous glands are derived from duct epithelium, which seems to have the ability to give origin to them. This ability probably can best be explained on the basis of a latent potentiality of differentiation in the adult epithelium. The existence of this potentiality is likely because, as Gruenwald[10] stated, "latent developmental potencies and lability of determination and differentiation are not, as early investigators believed, peculiarities of embryonic cells." If this latent potentiality of the duct epithelium is accepted, it would be easy to understand its differentiation into sebaceous

glands since both parotid and sebaceous glands are developed from the ectoderm.[11-13] Whether or not this explanation is accepted, it is evident that the duct epithelium can give origin to sebaceous glands in normal parotid glands as well as in those which are the site of neoplasia. Moreover, the latter group seems to have a greater tendency toward such differentiation, as appears to be substantiated (1) by the occurrence of sebaceous glands in greater numbers and in a more advanced state of development in such parotid glands than in those considered to be normal; (2) by the frequency with which sebaceous glands were seen in parotid glands which had given origin to tumors of the muco-epidermoid type, in which it has been observed that the duct epithelium may undergo transformation into mucus-forming cells and also into cells capable of showing different stages of transformation, varying from the normal type cell of the duct epithelium to cornifying squamous elements; and (3) by the occurrence of sebaceous glands in two parotid glands that were the site of pure adenomas, tumors that in themselves may be considered as the direct result of the latent potentiality of the glandular epithelium to undergo differentiation into cells of a new type.[1] It also appears that the epithelium of the parotid gland seems to be more liable to undergo differentiation than that of the submaxillary gland, as is shown by the greater incidence of sebaceous glands in the former. This is further proved by the fact that Hamperl[2] found only 4 instances of sebaceous-gland-like transformation in submaxillary glands in a study of salivary glands in 85 persons, while in the material of the present report this was observed in 28 parotid glands obtained from 51 individuals. From what has been said, it seems logical to think that an epithelium, in which an exaggerated latent potentiality of differentiation exists, is prone to give origin not only to normal structures, but also to tumors in which the cell component may resemble various epithelial elements of ectodermal origin, such as those sometimes found in salivary gland tumors. Thereby are explained some findings which have been noticed repeatedly in the mixed tumors of the salivary glands, such as the presence of squamous epithelium[14-17] and keratohyalin, the resemblance of some salivary gland tumors to the basal cell carcinoma of the skin,[16-20] to the adamantinomas,[20-22] and also to some tumors occurring in relation to the skin but with characteristics similar to mixed salivary gland tumors.[23]

Summary

Sections from 100 normal parotid glands and from 133 parotid glands which were the site of tumors were studied to find out (1) if structures resembling sebaceous gland are present in normal parotid glands, (2) if

such structures are present in parotid glands that are the site of tumor, and (3) if the presence of sebaceous glands might have some significance in the explanation of tumors occurring in the salivary glands.

From this study, the following results were obtained. Structures which could not be differentiated from sebaceous glands, measuring from 50 to 350 μ in diameter, were seen to originate from intercalated and striated ducts which ended blindly. Such sebaceous glands were present in 28 per cent of the normal parotid glands and in 24.8 per cent of the parotid glands in which tumors had occurred. Their occurrence was not related to sex, age, or state of atrophy of the parotid gland. Sebaceous glands were more numerous and in a more advanced state of development in the parotid glands in which tumors had occurred.

Sebaceous glands were associated with 17 mixed tumors, 8 muco-epidermoid tumors, 6 carcinomas, and 2 adenomas. There appears to be a high incidence of sebaceous glands in parotid glands in which muco-epidermoid tumors, carcinomas, and adenomas develop. There seems to be some evidence of greater susceptibility of the parotid epithelium to undergo differentiation as compared with that of the other salivary glands, which gives a possible explanation for the greater frequency of tumors in the parotid glands.

It is reasonable to believe that the sebaceous glands occurring in the submaxillary and parotid salivary glands arise from duct epithelium owing to the presence of latent potentiality of differentiation in that epithelium. This aids in developing a possible explanation for the origin of some of the components of the mixed tumors.

REFERENCES

1. Meza-Chávez, L. Oxyphilic granular cell adenoma of the parotid gland (oncocytoma). Report of five cases and study of oxyphilic granular cells (oncocytes) in normal parotid glands. *Am. J. Path.*, 1949, **25**, 523–547.

2. Hamperl, H. Beiträge zur normalen und pathologischen Histologie menschlicher Speicheldrüsen. *Ztschr. f. mikr.-anat. Forsch.*, 1931, **27**, 1–55.

3. Hartz, P. H. Development of sebaceous glands from intralobular ducts of the parotid gland. *Arch. Path.*, 1946, **41**, 651–654.

4. Río Hortega, P. El método del carbonato argéntico. *Arch. histol. norm. y pat.*, 1942, **1**, 165–205.

5. Stewart, F. W., Foote, F. W., and Becker, W. F. Muco-epidermoid tumors of salivary glands. *Ann. Surg.*, 1945, **122**, 820–844.

6. Stein, I., and Geschickter, C. F. Tumors of the parotid gland. *Arch. Surg.*, 1934, **28**, 492–526.

7. Benedict, E. B., and Meigs, J. V. Tumors of the parotid gland. *Surg., Gynec. & Obst.*, 1930, **51**, 626–647.

8. Margolies, A., and Weidman, F. Statistical and histologic studies of Fordyce's disease. *Arch. Dermat. & Syph.*, 1921, **3**, 723–742.

9. Sutton, R. L. Diseases of the Skin. C. V. Mosby Co., St. Louis, 1928, ed. 7, p. 1357.

10. Gruenwald, P. A. A note on the so-called undifferentiated and embryonic cells. *Arch. Path.*, 1943, **36**, 190–194.

11. Schulte, H. v. W. The Development of the Human Salivary Glands. In: Studies in Cancer and Allied Subjects. George Crocker Special Research Fund, Columbia University Press, New York, 1913, **4**, 25–72.

12. Patten, B. M. Human Embryology. The Blakiston Co., Philadelphia, 1946, 776 pp.

13. Arey, L. B. Developmental Anatomy. A Textbook and Laboratory Manual of Embryology. W. B. Saunders Co., Philadelphia, 1946, ed. 5, 616 pp.

14. McFarland, J. Adenoma of the salivary glands, with a report of a possible case. *Am. J. M. Sc.*, 1927, **174**, 362–378.

15. Leroux, R., Leroux-Robert, J., and Dillange, P. Étude analytique des éléments constitutifs des tumeurs dites mixtes des glandes salivaires. *Bull. Assoc. franç. p. l'étude du cancer*, 1933, **22**, 490–505.

16. Harvey, W. F., Dawson, E. K., and Innes, J. R. M. Debatable Tumours in Human and Animal Pathology. Oliver & Boyd, London, 1940, pp. 17–26.

17. Dockerty, M. B., and Mayo, C. W. Primary tumors of the submaxillary gland with special reference to mixed tumors. *Surg., Gynec. & Obst.*, 1942, **74**, 1033–1045.

18. Krompecher, E. Zur Histogenese und Morphologie der Mischgeschwülste der Haut sowie der Speichel- und Schleimdrüsen. *Beitr. z. path. Anat. u. z. allg. Path.*, 1908, **44**, 51–87.

19. Krompecher, E. Über die Beziehungen zwischen Epithel und Bindegewebe bei den Mischgeschwülsten der Haut und der Speicheldrüsen und über das Entstehen der Karcinosarkome. *Beitr. z. path. Anat. u. z. allg. Path.*, 1908, **44**, 88–149.

20. Hemplemann, L. H., Jr., and Womack, N. A. The pathogenesis of mixed tumors of the salivary gland type. *Ann. Surg.*, 1942, **116**, 34–42.

21. Sheldon, W. H. So-called mixed tumors of the salivary glands. *Arch. Path.*, 1943, **35**, 1–20.

22. Lang, F. J. Sogenannte Mischgeschwülste der Speicheldrüsen. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. J. Springer, Berlin, 1929, **5**, Pt. 2, 140–187.

23. Li, P. L., and Yang, C. An inquiry into the origin of the mixed tumors of the salivary glands, with reference to their embryonic interrelationships. *Am. J. Cancer*, 1935, **25**, 259–272.

[*Illustrations follow*]

DESCRIPTION OF PLATES

All photomicrographs were obtained from slides stained with hematoxylin and eosin, unless otherwise stated.

PLATE 96

FIG. 1. Case 18 of Table II. Sebaceous gland arising in a branch of a striated duct. Of note is the holocrine secretion falling into the lumen of the duct. × 375.

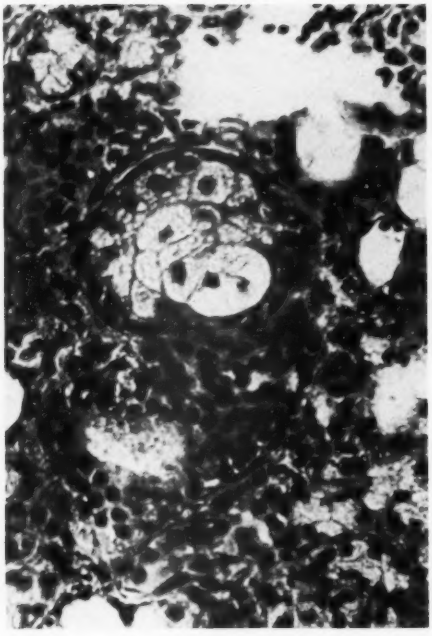
FIG. 2. Sebaceous gland in the parotid gland of the oldest person from group 1, case 27 of Table II. × 375.

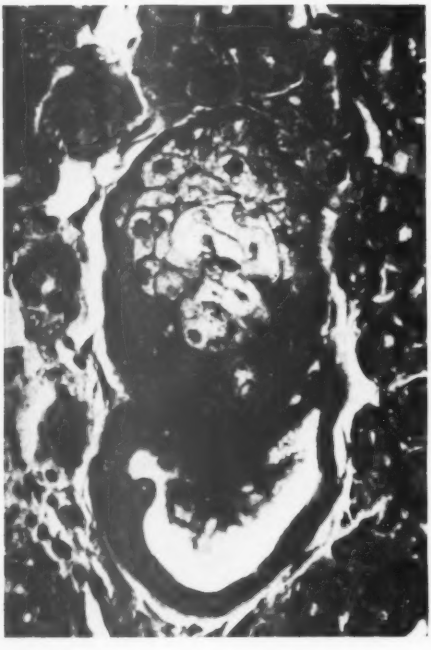
FIG. 3. Sebaceous gland in the youngest individual of group 1, case 16 of Table II. × 375.

FIG. 4. Sebaceous gland in one of the cases of adenoma of the parotid gland (case 12 of Table II). Of note is the holocrine secretion in the lumen of the duct. × 375.

1

3





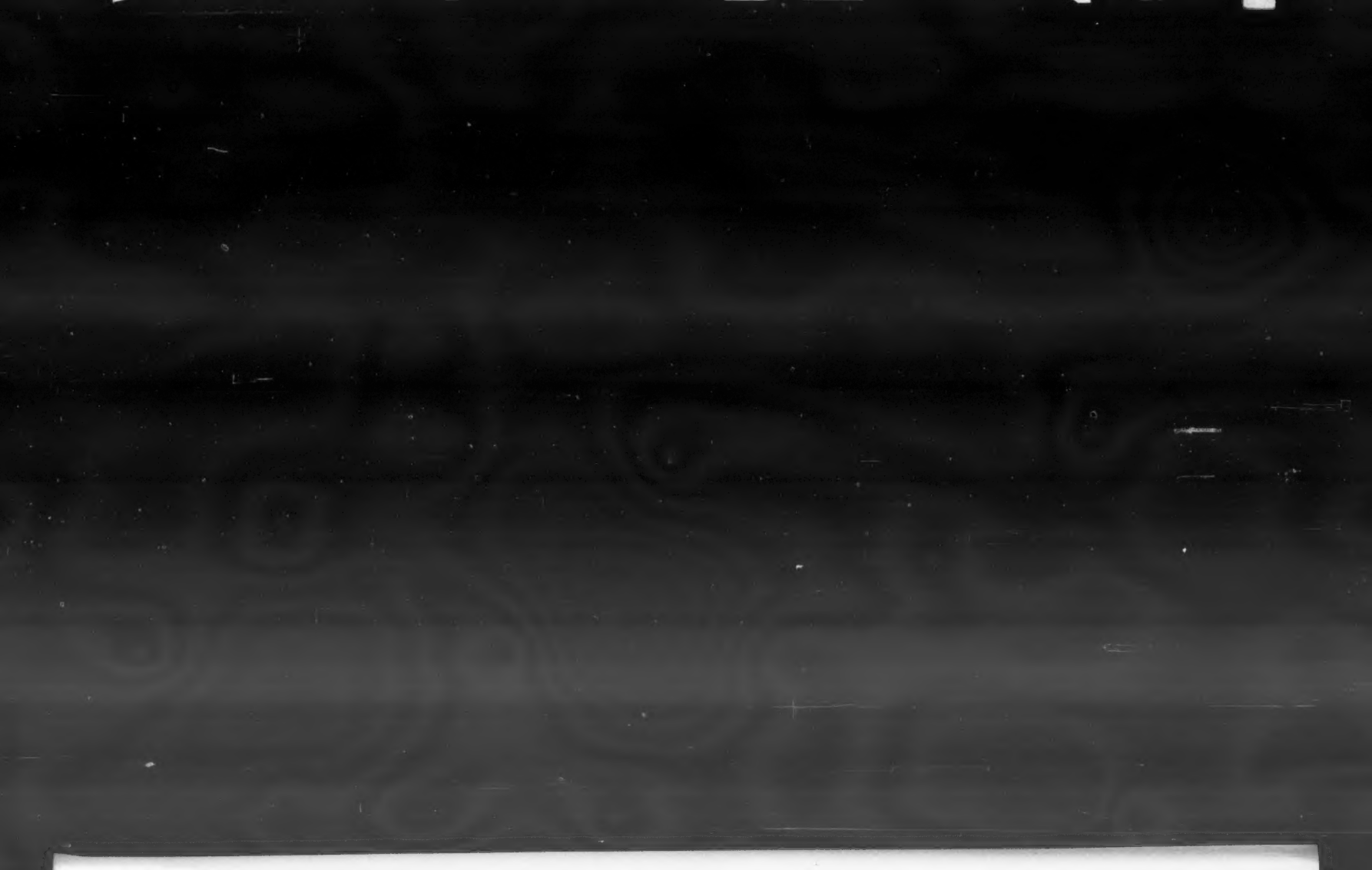
Meza-Chávez Sebaceous Glands in Parotid Glands

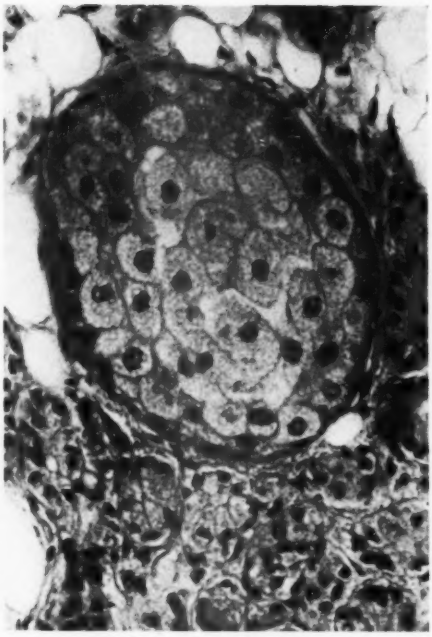
PLATE 97

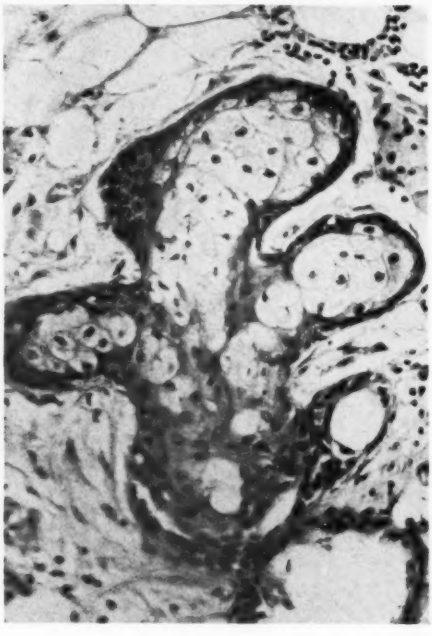
FIGS. 5 and 6. Sebaceous glands in a normal parotid gland (case 12 of Table III). Of note is the larger size of the nuclei of the sebaceous glands in comparison to the acinar and ductal cells. × 375.

FIG. 7. Sebaceous gland from another normal parotid gland (case 9 of Table III). There is a mitotic figure in a cell at the periphery of the alveolus. × 375.

FIG. 8. Simple branched sebaceous gland from a case of parotid adenoma, case 12 of Table III. × 375.







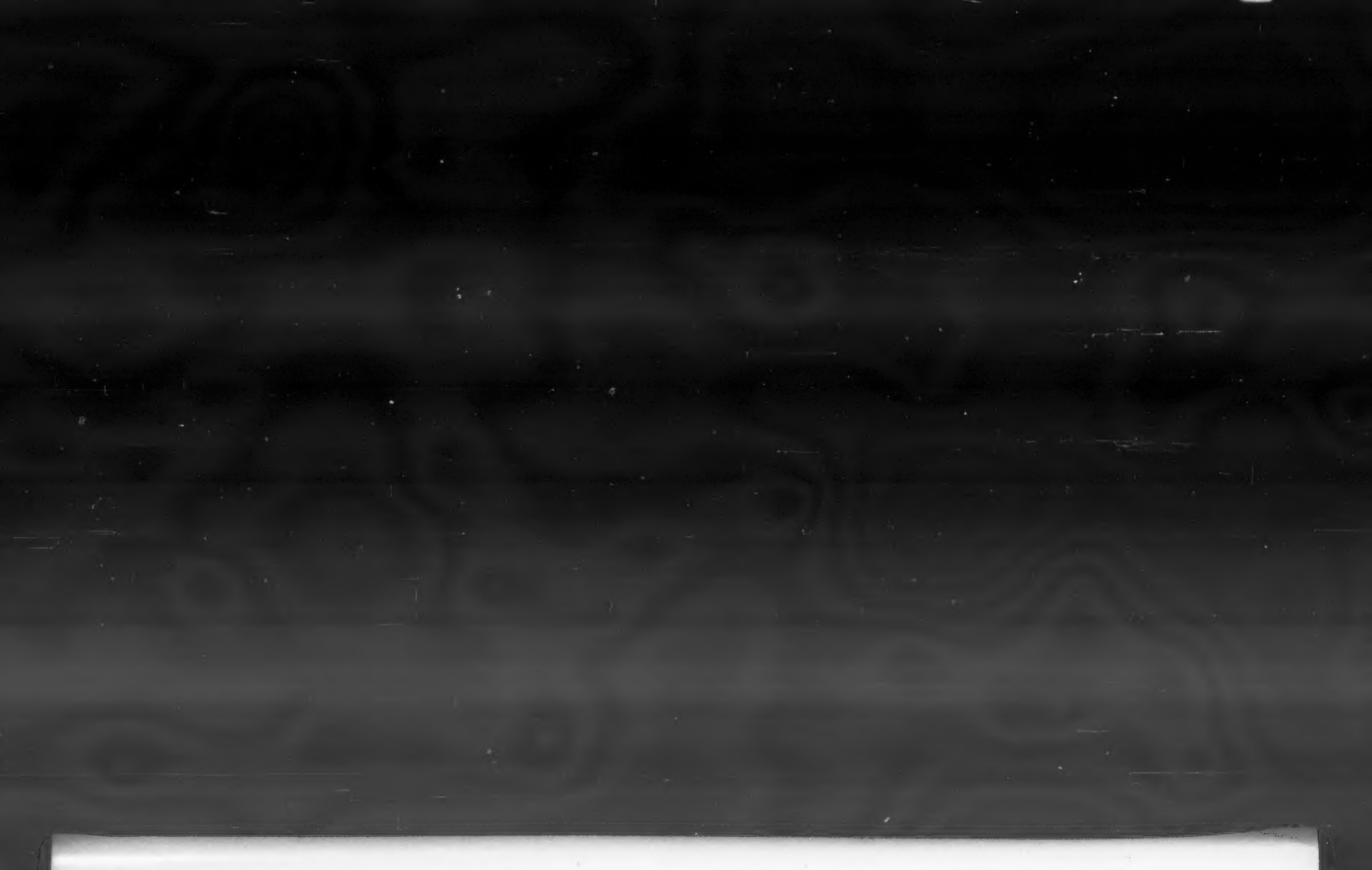
Meza-Chávez Sebaceous Glands in Parotid Glands

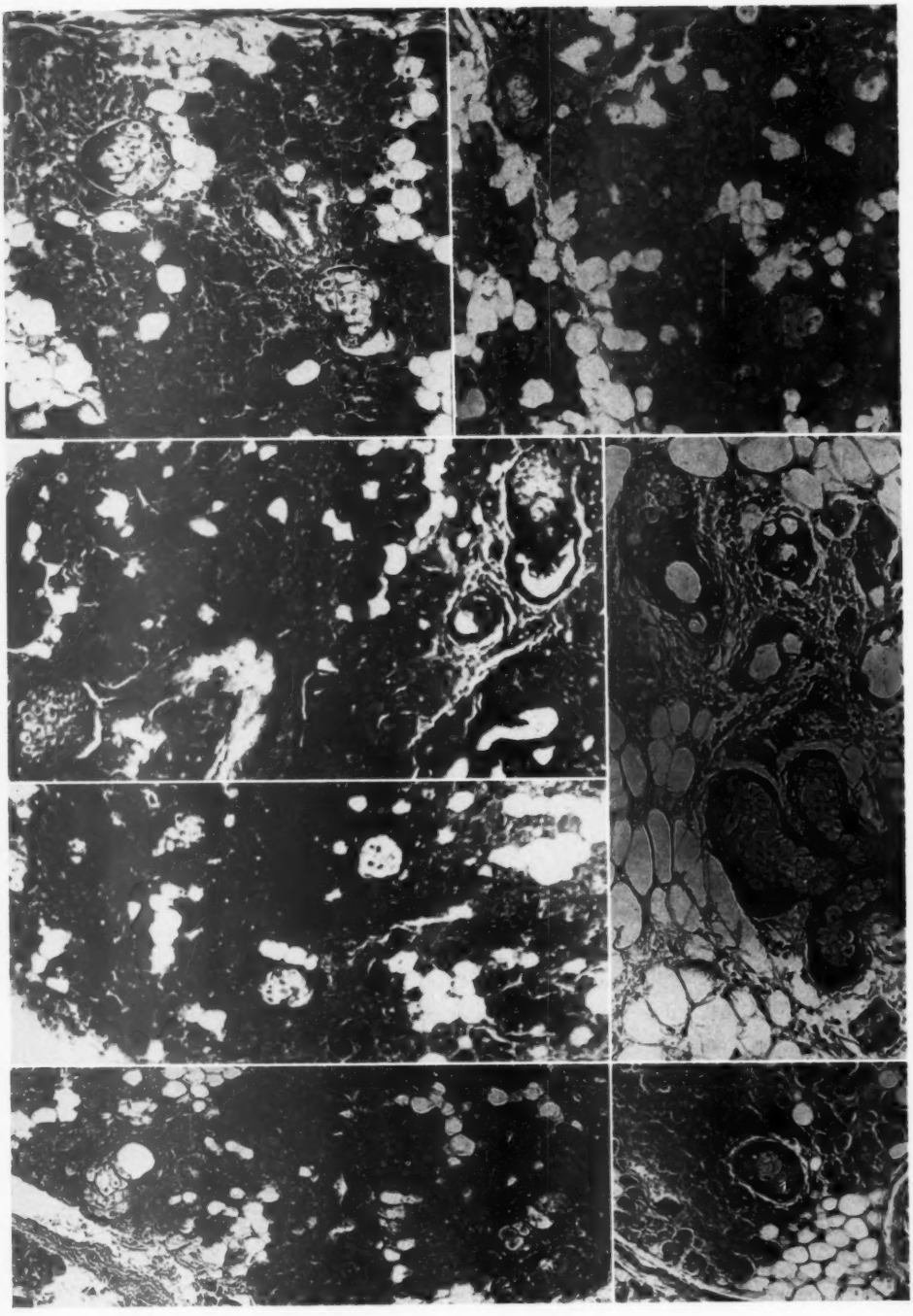
PLATE 98

FIGS. 9 to 13. Parotid glands from group 1 in which two or more sebaceous glands were seen in a single microscopic field (cases 18, 27, 16, 17, and 12, respectively, of Table II). × 115.

FIG. 14. Parotid gland from group 2 in which two sebaceous glands are seen in one microscopic field (case 7 of Table III). × 115.

FIG. 15. Early development of sebaceous glands in a normal parotid gland (case 13 of Table III). × 115.





Meza-Chávez Sebaceous Glands in Parotid Glands

PLATE 99

FIGS. 16 to 18. Three sections from a sebaceous gland arising from an intercalated duct which ends blindly (case 10 of Table III). × 115.

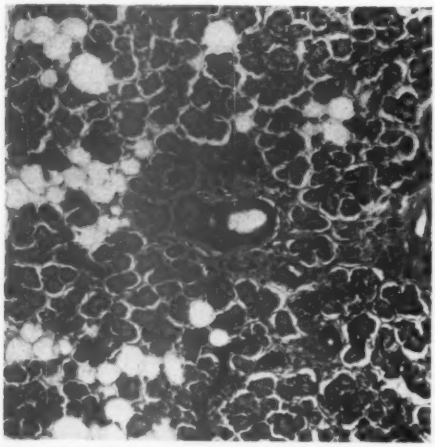
FIGS. 19 and 20. Sebaceous glands from normal parotid glands, with lymphocytic infiltration around them (cases 13 and 15 of Table III). × 115.

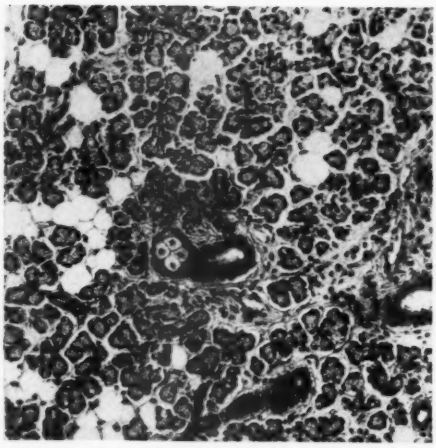
FIG. 21. Frozen section from a normal parotid gland showing a sebaceous gland (case 9 of Table III). Hortega's silver impregnation and scharlach R stains. × 115.

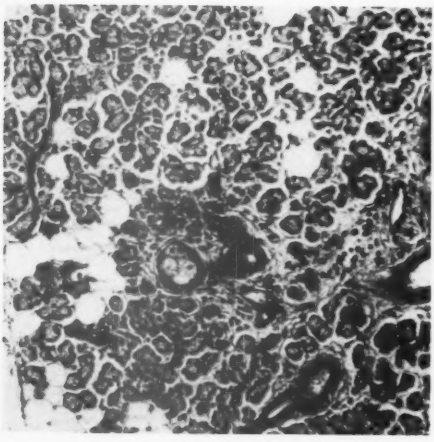
6

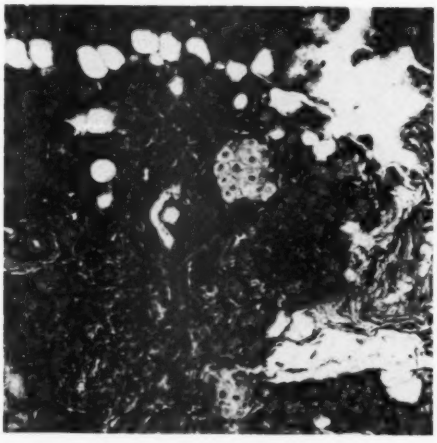
3

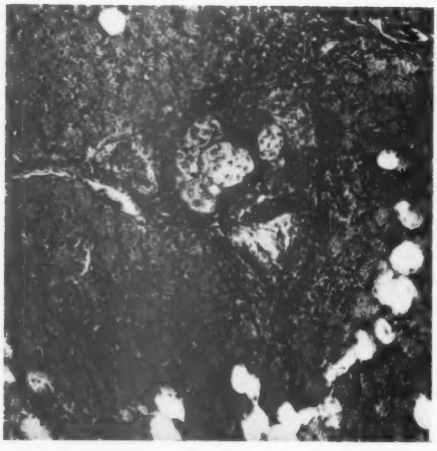
0

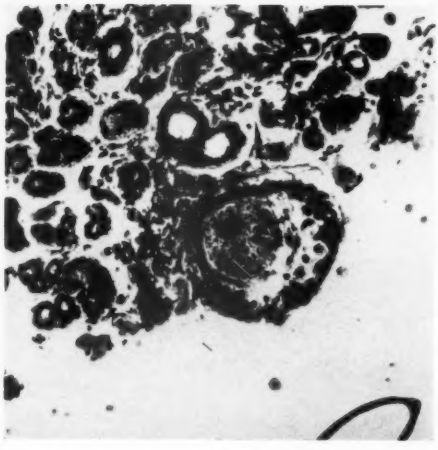












Meza-Chávez Sebaceous Glands in Parotid Glands

COMBINED LIVER CELL AND BILE DUCT CARCINOMA*

RAYMOND A. ALLEN, M.D., and JAMES R. LISA, M.D.

(From the Laboratory of Pathology, City Hospital, Welfare Island, Department of Hospitals, New York 17, N.Y.)

Carcinomas of the liver displaying features indicating origin from both liver cells and bile ducts have been observed infrequently. They have been reported under various terms: mixed tumors,[1,2] duplex type,[3] intermediate type,[3,4] carcinoma of dual origin,[5] cholangiohepatoma.[6] The first case so designated appears to be that of Wells.[7] Others have been recorded by Goldzieher,[8] 1 case; Tramontano and Fittipaldi,[9] 2 cases; Koster and Kasman,[3] 1 case; Rasario,[10,11] 2 cases; Bonne,[4] 1 case; and Cabot case no. 26441.[12] Gustafsen,[5] reporting on 62 cases of primary carcinoma of the liver, mentioned 2 of intermediate type. In the reports of primary liver cell carcinoma by Prescott,[13] Moon,[14] Larson,[15] and Neumann,[16] the data were very suggestive that the bile ducts participated in the malignant process. This communication is a report of 5 additional cases, found among 35 cases of primary carcinomas of the liver in 8175 consecutive autopsies performed at City Hospital, Welfare Island, between May 1, 1911, and May 1, 1948.

Usually a sharp distinction can be drawn between the two principal forms of primary carcinoma of the liver. Microscopically, Winternitz[17] distinguished between them on the bases of cell type, architectural pattern, and stromal reaction. These criteria generally have been employed by subsequent writers. Typically, liver cell carcinoma is comprised of large polygonal cells with a moderate amount of pink granular cytoplasm and large hyperchromatic vesicular nuclei with one or more prominent eosinophilic nucleoli. The cells are arranged in trabeculae along a capillary sinusoidal bed. The resemblance to normal liver tissue may be, and often is, unmistakable. The cells may contain bile. Giant cells are said to be more characteristic of hepatocellular carcinoma, although they occur infrequently. Kupffer cells are likewise said to be a distinguishing feature. In contradistinction, carcinoma derived from bile ducts consists of irregular cuboidal or columnar cells, sometimes showing stratification, with scanty basophilic cytoplasm and small hyperchromatic nuclei. There is usually a large amount of dense connective tissue in which the epithelial cells are arranged in irregular cords and duct-like formations. In appearance they do not differ from scirrhous tubular adenocarcinoma of other organs.

Certain peculiarities, however, serve to confuse one type with the

* Received for publication, July 29, 1948.

other, and in carcinoma of double nature, these features must be evaluated with even greater caution. Since the great majority of liver carcinomas occur with cirrhosis, the structural features of this condition must be carefully borne in mind. As noted by Hoyne and Kernohan,[18] the condensation of bile ducts and connective tissue in cirrhotic livers accompanying primary liver cell carcinoma should not be interpreted as a mixed neoplasm. It has been our experience also that this proliferation is sometimes so intense that it approaches a true adenoma and may mimic neoplasia, particularly if an inflammatory cellular reaction is absent. Nodular hyperplasia of the liver cells may be so marked that it can be distinguished from true neoplasia only with difficulty. Ewing,[2] Cunningham,[19] Winternitz,[17] and many others have emphasized the fact that liver cell carcinomas may produce structural features resembling bile ducts. In addition, degenerative and metaplastic changes may alter the appearance of the tumor so as to make its positive identification difficult. Thus, central necrosis of columns of carcinomatous liver cells may simulate bile ducts.

Since the majority of primary carcinomas arise in cirrhotic livers and may be unicentric or multicentric in origin, three combinations of tumors of double nature are possible. (1) Separate neoplastic masses may be comprised entirely of the liver cell type on the one hand, and of the bile duct type on the other. (2) Contiguous masses, each of different character, may mingle as they grow. (3) Individual masses may display both features so intimately associated that they can be interpreted only as arising from the same site. Each of these possibilities is illustrated by authentic cases in the literature and in our material.

Separate nodules of hepatocellular and bile duct character were present in case 1 (autopsy 3542). The liver, weighing 3600 gm., was studded with hard white masses and did not appear cirrhotic. The white masses consisted of characteristic bile duct carcinoma—ducts lined by malignant cells lying in abundant dense fibrous tissue. This was the predominant finding. The cirrhotic process in the remainder of the liver was mild and many nodules had characteristic malignant liver cells with the sinusoidal bed preserved. Metastases of ductal character were present in the hilar and upper abdominal lymphatics. A thrombus showing a mixture of neoplastic ductal and hepatic cells was present in a hepatic vein; pulmonary metastases were similar.

Contiguous carcinomatous nodules of different character were present in case 2 (autopsy 5305). The liver, which weighed 5150 gm., was extremely cirrhotic and studded with soft yellow, and hard white,

masses. The nodules of hepatic cell type predominated and varied considerably in appearance. Some had the familiar cords of large granular cells separated by a sinusoidal bed. In others the cells were extremely anaplastic, small, and mimicked cells of lymphoid nature. The bile duct nests were very scirrhous. Although the two types were frequently contiguous, there was no distinct intermingling. The metastases, of bile duct nature, were confined to the hilar and upper abdominal nodes; there were no metastases of liver cell type.

The malignant types were in more intimate association in case 3 (autopsy 254). The liver was enlarged, cirrhotic, and had many masses of variable size and appearance. Most of the nodules were of liver cell character, Kupffer cells were abundant, and both hepatic and portal venous radicles had similar tumor thrombi. One nodule (Figs. 1 and 2) had groups of large cuboidal cells with opaque cytoplasm at its periphery, in sharp contrast to the adjacent malignant hepatic cells which had a lighter, finely granular cytoplasm containing fine bile pigment. Near by, within the fibrous tissue of the portal area, were ductal structures lined chiefly with large cuboidal opaque cells, intermingled with somewhat larger cells having more abundant, finely granular cytoplasm, similar to the cells deep in the nodule. There appeared to be transitions between the two kinds of cells. Bile canaliculi could not be demonstrated. Metastases were absent.

Extraordinary intermingling of the carcinomas occurred in the remaining two cases. The liver in case 4 (autopsy 5490) was extremely cirrhotic, weighed 1150 gm., and had a soft yellowish brown mass 1.5 cm. in diameter in the right lobe. Microscopically, this mass consisted of nodules containing carcinomatous liver cell cords and bile ducts (Fig. 3) separated by thin-walled capillaries. The cords had large cells with finely granular, faintly acidophilic cytoplasm. Bile canaliculi were not seen. The ducts had large opaque basophilic cells. An occasional duct was lined by cells of both types (Fig. 4), each distinct, sometimes adjacent, and apparently without transitional forms. The nodules were free of fibrosis. One small nodule of pure liver cell type was present. There were no metastases.

The liver of case 5 (autopsy 7262) weighed 4200 gm., was intensely cirrhotic, and had a variety of colors in the nodules—yellow, gray, green, tan, and white. Most of the nodules were similar to those of case 4, showing an intimate mingling of malignant ducts and liver cell cords. In some areas where duct formation was more prominent, extremely fine fibrosis was present. Transitional forms between the cell types was evi-

dent, especially where they lined ductal structures. Some nodules showed a single malignant type, either liver cell or bile duct. One carcinomatous duct showed marked papillation. The metastases were of mixed nature and were present in the aortic, tracheobronchial, and right renal lymph nodes and in the right adrenal gland.

The views concerning the specificity of liver and bile duct cells are at once controversial and contradictory. Embryologically, it is well established that liver cells and bile ducts have a common origin from the primitive gut.[20] For the normal liver, McIndoe[21] and others have described a gradual transition from the cuboidal epithelium of the bile ducts to the large liver cells, and the presence of an intermediate epithelial type. Rindfleisch[22] believed that cells of bile ducts may originate from the parenchymal cells. On the other hand, in regenerating liver associated with cirrhosis, MacCallum[23] saw what he believed to be new liver cells arising from bile duct epithelium. Muir[24] likewise believed that in certain cases bile duct epithelium may be transformed into hepatic cells. Lucké[25] observed the differentiation of hepatic cells from biliary epithelium. That either cell gives rise to the other has been challenged by Counseller and McIndoe,[26] Fox and Bartels,[27] and Mallory,[28] who contended that each cell type remains specific and may reproduce only its own kind.

There is no less contradiction regarding the interpretation as to histogenesis of the findings in mixed tumors of the liver. Ewing[29] held that most neoplasms originate from adult cells and that the anaplasia of tumors bears no relationship to embryonal reversion. He expressed the opinion[2] that the mixed tumor had either a dual origin or had possibly arisen from cells which earlier assumed the characteristics of pseudo-bile ducts. Counseller and McIndoe[26] believed that each cell type gives rise to its corresponding tumor entity, and never to the other. Wells[7] believed that the tumor he reported had origin from both liver cells and bile ducts. Goldzieher[8] considered that undifferentiated liver cells may assume the form of pseudo-bile ducts, from which bile duct carcinoma may arise. Moon[14] noted what he believed to be liver cells originating from proliferating bile ducts in a case of primary liver cell carcinoma. Our own material fails to throw light on this question. In one case of intimate association of the two types of malignant cells, there appeared to be transitional forms. In the other, each cell seemed to remain specific.

Summary and Conclusions

Five cases of combined primary liver cell and bile duct carcinoma illustrate three combinations of tumors of double nature: (1) separate

nodules of hepatocellular and bile duct carcinoma; (2) contiguity with intermingling; (3) intimate association due to origin from the same focus. The present conflicting views of the relationship of hepatic and ductal cells in the histogenesis of these neoplasms cannot be resolved on the basis of this material.

REFERENCES

1. L'Esperance, E. Atypical hemorrhagic malignant hepatoma. *J. M. Research,* 1915, **32**, 225–249.
2. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia & London, 1940, ed. 4, p. 740.
3. Koster, H., and Kasman, L. P. Primary duplex liver carcinoma. *Am. J. Surg.,* 1932, **17**, 237–241.
4. Bonne, C. Cancer and human races. *Am. J. Cancer,* 1937, **30**, 435–454.
5. Gustafsen, E. G. An analysis of sixty-two cases of primary carcinoma of the liver based on 24,400 necropsies at Bellevue Hospital. *Ann. Int. Med.,* 1937–38, **11**, 889–900.
6. Warvi, W. N. Primary neoplasms of the liver. *Arch. Path.,* 1944, **37**, 367–382.
7. Wells, H. G. Primary carcinoma of the liver. *Am. J. M. Sc.,* 1903, **126**, 403–417.
8. Goldzieher, M. A. Über multiple Carcinome, ein Beitrag zur Frage der Histogenese des Lebercarcinoms. *Virchows Arch. f. path. Anat.,* 1928, **267**, 326–338.
9. Tramontano, V., and Fittipaldi, C. Sui cosidetti tumori a cellule giganti. *Tumori,* 1930, **16**, 501–518.
10. Rasario, G. M. Adeno-carcinoma epatocellulare primitivo del fegato. *Riv. di clin. med.,* 1934, **35**, 767–786.
11. Rasario, G. M. Ulteriore contributo allo studio clinico ed anatomo-patologico della cancro-cirrosi del fegato. *Clin. med. ital.,* 1937, **68**, 807–817.
12. Cabot case no. 26441. Primary cancer of the liver, combined type: adenocarcinoma and hepatoma. *New England J. Med.,* 1940, **223**, 731–734.
13. Prescott, W. H. Seven cases of primary carcinoma of the liver. *Med. & Surg. Rep. Bost. City Hosp.,* 1895, **6**, 245–267.
14. Moon, V. H. Primary carcinoma of liver with metastasis to bone. *Arch. Path.,* 1929, **8**, 938–943.
15. Larson, C. P. Multiple liver-cell carcinoma (primary type). Report of a case. *Northwest Med.,* 1937, **36**, 388–391.
16. Neumann, M. A. Primary carcinoma of the liver with metastases to bone. *Am. J. Path.,* 1944, **20**, 895–909.
17. Winternitz, M. C. Primary carcinoma of the liver. *Johns Hopkins Hosp. Rep.,* 1916, **17**, 143–184.
18. Hoyne, R. M., and Kernohan, J. W. Primary carcinoma of the liver. *Arch. Int. Med.,* 1947, **79**, 532–554.
19. Cunningham, R. M. Primary carcinoma of the liver. A clinical-pathologic report of 14 cases at the University Hospital from 1927 to 1943. *Bull. School Med. Univ. Maryland,* 1943–44, **28**, 61–79.
20. Lewis, F. T. The Development of the Liver. In: Keibel, F., and Mall, F. P. (eds.) Manual of Human Embryology. J. P. Lippincott Co., Philadelphia & London, 1912, **2**, 403–428.
21. McIndoe, A. H. The structure and arrangement of the bile canaliculi. *Arch. Path.,* 1928, **6**, 598–614.

22. Rindfleisch, E. Microscopische Studien über das Leberadenoid. *Arch. Heilk.*, 1864, **5**, 395–401. (Cited by Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia & London, 1940, ed. 4, p. 751.)
23. MacCallum, W. G. Regenerative changes in cirrhosis of the liver. *J. A. M. A.*, 1904, **43**, 649–654.
24. Muir, R. On proliferation of the cells of the liver. *J. Path. & Bact.*, 1907–08, **12**, 287–305.
25. Lucké, B. The pathology of fatal epidemic hepatitis. *Am. J. Path.*, 1944, **20**, 471–593.
26. Counseller, V. S., and McIndoe, A. H. Primary carcinoma of the liver. *Arch. Int. Med.*, 1926, **37**, 363–387.
27. Fox, R. A., and Bartels, G. W. Primary carcinoma of the liver. Report of three cases. *Arch. Path.*, 1928, **6**, 228–239.
28. Mallory, F. B. Cirrhosis of the liver; five different types of lesions from which it may arise. *Bull. Johns Hopkins Hosp.*, 1911, **22**, 69–75.
29. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia & London, 1940, ed. 4 pp. 12–13.

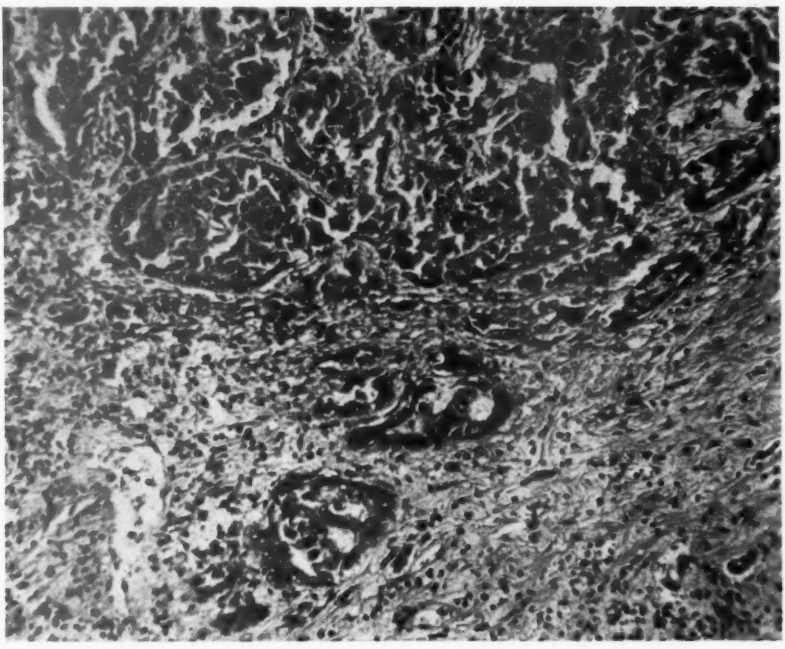
DESCRIPTION OF PLATES

PLATE 100

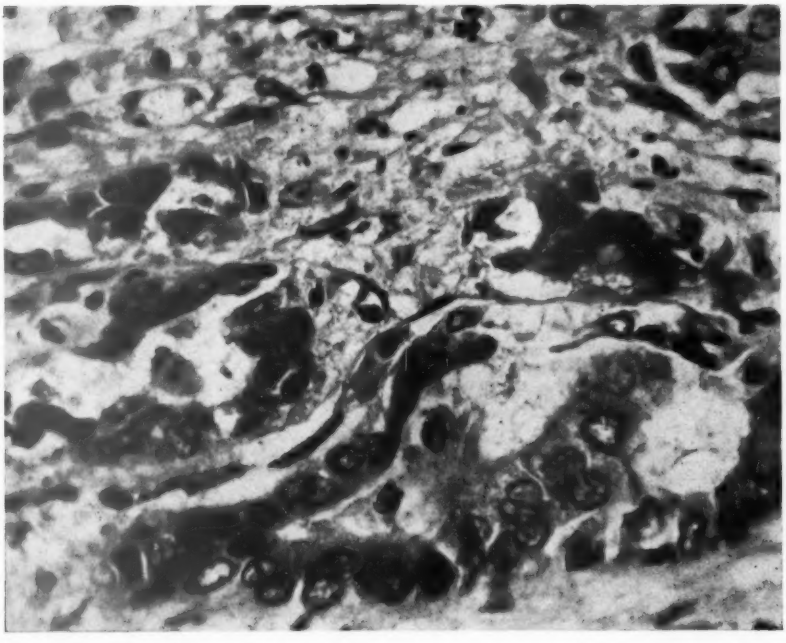
FIG. 1. Case 3. A nodule showing chiefly a neoplasm of liver cell type. At the periphery, some groups have a zone of more opaque cells suggestive of the bile duct type. In the surrounding fibrous tissue there are ductal structures with bile duct and liver cell epithelium. × 135.

FIG. 2. Case 3. A duct is lined partly by large cuboidal opaque cells and partly by large granular cells of hepatic parenchymal character. Cells of the two types apparently have transitional forms. × 400.

1



2



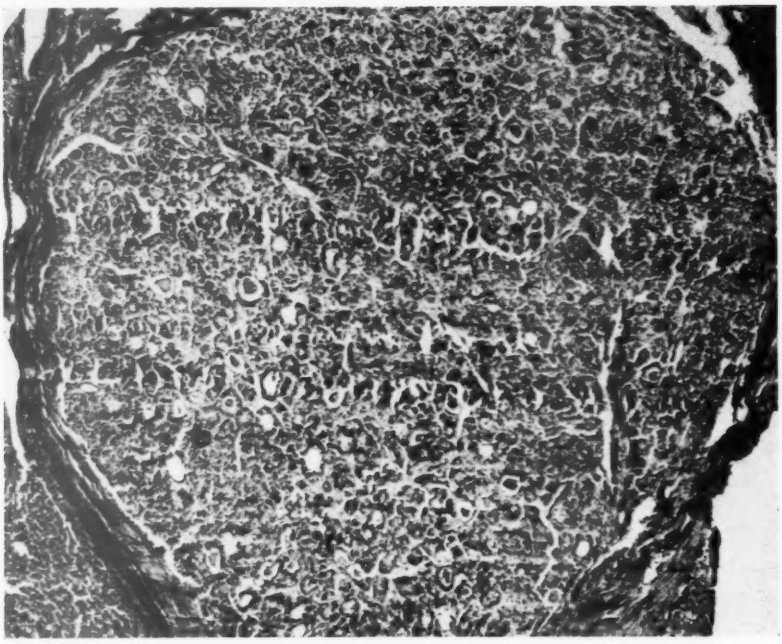
Allen and Lisa Combined Liver Cell and Bile Duct Carcinoma

PLATE 101

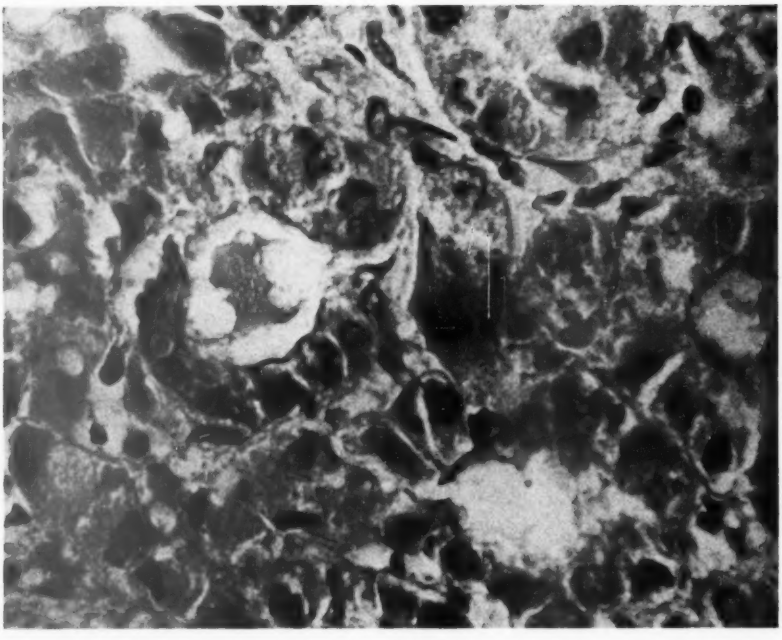
FIG. 3. Case 4. A nodule surrounded by a fibrous wall has an intimate mingling of ducts and cords. × 45.

FIG. 4. Case 4. The ducts are lined chiefly by large cuboidal or columnar opaque cells, a more granular cell occasionally being present, as in the duct on the left. Other small nests are composed of large granular cells only, some of giant size. × 400.

3



4



Allen and Lisa Combined Liver Cell and Bile Duct Carcinoma

PATHOGENESIS OF ULCERATIVE COLITIS*

SHIELDS WARREN, M.D., and SHELDON C. SOMMERS, M.D.
(From the New England Deaconess Hospital Laboratory of Pathology, Boston 15, Mass.)

Ulcerative colitis has been thoroughly reviewed clinically,[1-5] but no pathologic investigation has been made recently, perhaps because the predominating manifestations are those of nonspecific inflammation. The importance of this condition demands that it be better understood. In the hope of advancing the fragmentary knowledge of its evolution, a series of 120 surgically treated and 60 autopsied cases of ulcerative colitis has been reviewed. All autopsies available, and surgical cases from the 20-year period of 1927 to 1946 were included. The series comprised approximately two-thirds of all cases diagnosed as ulcerative colitis in this laboratory. Ulcerative colitis of specific etiology, such as tuberculosis, amebic or bacillary dysentery, lymphopathia venereum, vitamin deficiency, and uremia, was excluded. Cases lacking sufficient histologic material for detailed analysis were discarded.

A clinical classification of nonspecific ulcerative colitis has been provided by Bargen[1]: type 1, or thrombo-ulcerative colitis, attributed to diplostreptococcal infection; type 2, or regional colitis, affecting mainly the right colon; and type 3, or atypical ulcerative colitis, also termed "idiopathic." These distinctions do not lend themselves to close pathologic correlation. In the course of study of our cases, two distinct pathogenetic processes were observed which lead to ulceration. They are termed type A (vasculitis) and type B (crypt abscess). Half the cases gave no clue as to their pathogenesis and are called indeterminate.

INCIDENCE

Among chronic intestinal diseases, ulcerative colitis is relatively common.[1,2] Studies have shown either equal sex distribution,[5-7] or a preponderance of females.[8,9] Women made up 55 per cent (100) of the 180 cases being analyzed, and men 45 per cent. The type A (vasculitis) cases, 19 in number, included 15 women, and type B (crypt abscess) cases numbered 71, with 33 women and 38 men. The 90 indeterminate cases included 52 women and 38 men.

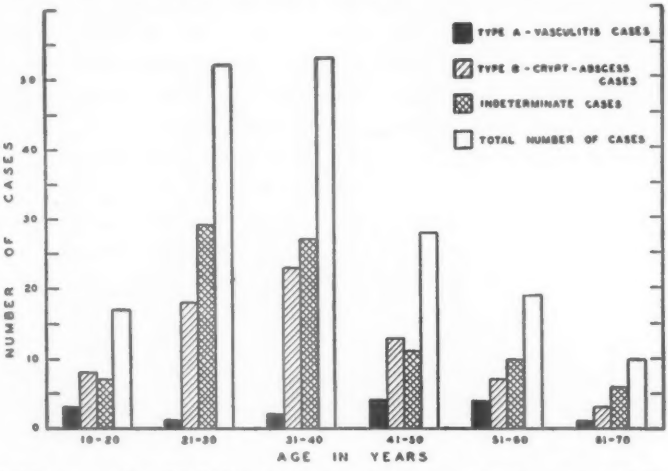
Ulcerative colitis is reported to occur largely in the twenties and thirties.[6,9] In the present series, 104 cases (58 per cent) fell in these decades, but the age range was 11 to 67 years. Cases in the newborn,[10,11] childhood,[12,13] and old age[14] also have been recorded. There were no significant fluctuations in the age incidence of type A (vasculitis) cases,

* Received for publication, August 7, 1948.

as shown in Text-Figure 1. Type B (crypt abscess) cases showed a peak incidence in the third and fourth decades, similar to that of cicatrizing enteritis. No additional significant information was provided by combining the analyses of sex and age.

Symptoms and Course

Diarrhea with blood and mucus, abdominal cramps, and tenesmus are the most important symptoms. Fever, weakness, tachycardia, malnutrition, and mental abnormalities often are prominent. Ulcerative colitis may have either a sudden or insidious onset,[15,16] and may pursue a fulminating,[17] slowly progressive, or irregular course with exacerbations



Text-Figure 1. Age distribution by decades of total number of cases and of the cases of each type of ulcerative colitis.

and remissions for many years.[6,8,15] Thirty years ago, 50 per cent of the patients died,[15] and collected series of over 1200 more recent cases[6,7,9,18,19] have an average mortality of 17.3 per cent. A few patients are cured, and the remainder are affected intermittently for life.[20] Clinically, ulcerative colitis tends to be a chronic irreversible disease, varying in extent, activity, and severity.[18,21,22]

Among our 60 fatal cases, a definite statement of the duration of ulcerative colitis was made in 54. Seven patients lived 2 months or less after onset, 12 lived from 2 to 6 months, and 3 lived from 7 to 12 months. Thus 22 patients (37 per cent) had histories of 1 year or less. The disease lasted from 1 to 5 years in 19 cases (32 per cent). There were 7 cases of 5 to 10 years' duration, 5 cases with 10 to 20 years of colitis, and 1 patient succumbed after 27 years. Symptoms of more than 5 years' duration

were present in 13 (22 per cent). Equal numbers of type A (vasculitis) and type B (crypt abscess) patients died within 1 year of onset.

Distribution

The extent and location of the lesions of ulcerative colitis are interesting. Clinically, the ulcers are said usually to begin and become most widespread in the rectum and sigmoid.[7,15,23,24] Accessibility to examination may contribute to overemphasis of this early ulcerative proctitis. It is often said that thereafter the ulcers gradually spread upward until

Table I

Distribution of Ulcers in Intestine, and Accompanying Conditions

Cases	Ileum	Colon					
		Cecum	Ascending	Transverse	Descending	Sigmoid	Rectum
45 autopsy	21	28	30	37	39	43	37
64 surgical	16	39	49	50	51	47	45
109 total	37(34%)	67(61%)	79(72%)	87(80%)	90(83%)	90(83%)	82(75%)

Segmental distribution: 2 cases
Right colon only: 3 cases

Appendix

Present 61 cases	Appendicitis:	Acute	1 case
		Recurrent acute or healing	4 cases
Absent 6		Ulcerated or chronic	12 cases
		Healed	10 cases
Negative 11 cases		Healed with partial obliteration	7 cases
		Healed with obliteration	8 cases

Accompanying Conditions

Mucosal polyp	8 cases
Multiple mucosal polyps	2 cases
Melanosis coli	3 cases
Meckel's diverticulum	4 cases
Other diverticula	3 cases
Calcified mesenteric lymph nodes	3 cases

the whole colon is involved. The right colon is ulcerated first in about 10 per cent of cases, and later the lower colon.[19,23] However, there is some doubt about these statements.[5,25] It may be that the colon usually is affected by the disease simultaneously, in part or as a whole, without subsequent spread.

Adequate data as to the distribution of the lesions were available in 109 of our 180 cases. The observations from autopsies are probably of greater value, since the surgical material tends to be influenced by clinical and technical factors. In 45 autopsies, the sigmoid was most often ulcerated, the descending colon next most frequently, and the cecum least commonly. No difference was found in the distribution of lesions in cases of the vasculitis type and of the crypt abscess type. The incidences are given in Table I. Extension of ulcerative colitis to the ileum in one-

third of the cases is about the frequency previously reported.[26] Ulcerative colitis clearly involved the appendix in one-fifth of our cases, and possibly affected it in another three-fifths. This is one of the exceptional diseases accompanied by chronic appendicitis.

Twenty-five female patients with total colectomies had aggregate lengths of large intestine averaging 86 cm., with a range of 56 to 116.5 cm. Thirty similar male cases had an average colon length of 99 cm., with extremes of 74 and 140 cm. The normal length of the large intestine is said to be approximately 150 cm.[27] In ulcerative colitis, as a result of contraction of scar tissue, the colon is thus shortened by about one-third.

Accompanying conditions, such as melanosis coli, Meckel's or other diverticula, calcified mesenteric lymph nodes, and mucosal polyps are listed in Table I. The incidences are comparable with those in the general population.[28-30] Neoplastic polyps were distinguished from inflammatory pseudopolyps by pathologic criteria described elsewhere.[31-33]

Gross Appearance

The dramatic picture of severe ulcerative colitis viewed from the mucosal surface is familiar to most physicians. The lining of the colon is deep red or purple due to marked hyperemia and congestion, and is wet and shining with blood and mucus. Petechial hemorrhages are scattered over the surface. The ulcers vary in number, size, shape, extent, and distribution. The smallest are punctate, and at times pus may be expressed from them; or ulcers may be so broad and coalescent that only a few irregularly shaped, elevated epithelial patches remain, and the mucosa is practically absent from part or all of the colon (Fig. 1). More frequently there are narrow, longitudinal, gutter-like depressions, three or four in number, extending through most of the length of the colon. These may overlie the muscular taeniae,[34] but are usually between them. A network of girdling ulcers connects the longitudinal gutters. Characteristically the submucosa is exposed but not penetrated, and its surface is shiny, red or gray. Soft, bulbous, edematous pseudopolypoid nodules are formed by the remaining mucosa, and these overhang and may entirely conceal the ulcers. Stretching or fixation of the colon will expose concealed ulcerations (Fig. 2).

During active progression of ulcerative colitis, the wall of the colon is irregularly thickened. Among 73 cases in which the maximum thickness was recorded, it averaged 0.6 cm. The range was 0.1 to 2.0 cm. Ulcers at times penetrate the muscle layers, usually in several places, and only subserosal connective tissue contains the fecal stream. In consistency the intestine then becomes as friable as wet paper.

A marked contrast is provided by the relatively unchanged external appearance of the unopened colon. The serosa is slightly edematous and congested. The mesentery contains scanty fat and numerous enlarged soft lymph nodes. The bowel lacks the normal sacculations and is slightly contracted, which is attributable to thickening of the submucosal and muscular layers.[8]

Roentgenologic study by barium enema gives additional information. In the early stage a marked spasm of the rectal ampulla is found,[35] which, if overcome, permits rapid filling of a negative-appearing colon. Later the mucosa presents a feathery pattern due to ulcerations and edema. Eventually, pseudopolyps may become recognizable. The tendency is for the colon to become narrowed and shortened, forming a stiff tube-like channel.[36] Margins of the diseased area are rarely sharply demarcated or segmental as in cicatrizing enteritis.

Proctoscopic observations reported by Buie[37] and Bargen[1] allow four stages of the developing disease to be distinguished: (1) hyperemia, (2) edema, (3) multiple, minute, superficial abscesses, and (4) coalescence with formation of large ulcers. Occasionally one may see (5) mucosal regeneration and healing.

The colon in a late stage of ulcerative colitis, when healing has followed medical treatment or surgical treatment such as ileostomy, appears quite different. The lumen is uniformly narrowed, with real or apparent overgrowth of mesenteric fat, which surrounds almost the entire outer surface. The circumference in 49 cases measured averaged 3.4 cm., with extreme values of 1.0 and 8.0 cm. This would indicate a lumen averaging 1.1 cm. in diameter. The mucosa is thin, with smooth and granular patches. It is usually tan or yellow with gray lymphoid aggregates, scattered pseudopolypoid tags, and punctate or longitudinal pitted and creased scars representing sites of healed ulcers. The bowel wall is indurated by submucosal fibrosis and muscular thickening. The serosa is slightly stiffened and opaque. Regional lymph nodes are small.

Gross distinctions can be made between the cases of type A (vasculitis) and type B (crypt abscess). Cases of type A are characterized by more severe, more extensive, and deeper ulcerations. Much of the thickness of the colonic wall is affected locally by a process similar to infarction elsewhere in the body. This is correlated with a higher mortality, since 12 of the 19 cases of this type form part of the autopsy group. Only 5 of the 19 developed pseudopolyps, which are an indication of long-continued superficial damage. Cases of the crypt abscess type correspond to the most common type of ulcerative colitis, as described grossly by Bargen[1] and others, and proctoscopically by Buie.[37] Pus can at times be ex-

pressed from superficial mucosal abscesses. Pseudopolyps were present in 49 of 71 cases. The mortality of colitis of this type is apparently less, since only 18 of the 71 cases are in the autopsy group. Among the 90 indeterminate cases, 9 were pathologically inactive, and 27 had pseudopolyps. The indeterminate group comprises exactly one-half in each instance of the total number of surgical cases and of the autopsy cases. Histologic observations form the basis of this classification.

Microscopic Findings

Changes in the mucosa and submucosa of ulcerative colitis are most important histologically. Epithelial continuity is interrupted by ulcers which tend to be multiple and shallow. Beneath the ulcers is a heavy infiltrate of lymphocytes, plasma cells, eosinophils, and macrophages, with very vascular granulation tissue. Inflammation may remain localized above the muscularis mucosae, but usually the inner half of the congested, edematous submucosa is involved also. Foci of perivascular and perineural chronic inflammation are observed in the outer submucosa, intermuscular septa, and subserosa. Pathologically, little is gained by separating ulcerative colitis into acute and chronic stages. Chronic inflammatory exudate, granulation tissue, and fibrosis are present very soon after the onset, and conversely, after years of the disease, foci of acute inflammation may still be found.

Type A (vasculitis) cases were so classified because of lesions of blood vessels, such as necrosis of the vascular walls, thrombosis, arteritis, phlebitis, periarteritis, or several of these abnormalities. Medium-sized submucosal arteries were affected uniformly, and in early stages the overlying mucosa appeared unchanged (Fig. 3). Subserosal or mesenteric vessels sometimes were similarly damaged. Thrombosis usually was present and the appearance often was identical with periarteritis nodosa or thrombo-angiitis obliterans except for the location in the intestine (Figs. 4 and 5). Interference with the local blood supply led to sloughing of part of the mucosa and damage to the wall beneath, which in the most severe cases resulted in gangrene and multiple perforations of the intestine. In later stages, hyaline vascular sclerosis with obliteration or recanalization of some large vessels was observed. Vasculitis was found in 19 cases (11 per cent) of our series, 15 being women, and 12 patients died. Bargen, in his microscopic studies of "thrombo-ulcerative colitis," described similar changes,[1] but he ascribed them to a more common, less severe variety of ulcerative colitis, clinically and grossly closely resembling the next type to be considered.

Type B (crypt abscess) colitis was initiated by the appearance of

polymorphonuclear leukocytes within the lumina of individual mucosal crypts just above their bases. A little eosinophilic mucoid secretion sometimes was mixed with the leukocytes or layered along the epithelium (Fig. 6). No abnormality of the epithelium was seen. As more polymorphonuclear leukocytes accumulated in an affected crypt, the base was distended, with flattening of the epithelium (Fig. 7). Eventually the crypt abscess ruptured at the lower end, spilling its contents into the submucosa. Purulent material dissected beneath the adjacent epithelium, which was ulcerated away (Fig. 8). Pus then escaped into the lumen of the bowel. Occasionally an abscess persisted in the submucosa, topped by a single layer of viable epithelium. Mucosal bridges were formed beneath which the purulent exudate flowed (Fig. 9). This undermining inflammation predisposed to the development of overhanging mucosal islands at the ulcer margins. In time, edema and chronic inflammation of the remaining mucosa produced localized pseudopolypoid projections. The lesions described were superficial and irritated the deeper layers of the colon without seriously damaging them. Fresh crypt abscesses appeared to occur in crops for a long time, periodically reactivating the partially healed colitis. Abscess formation was not encountered in the connective tissue of the lamina propria or submucosa except after rupture of crypt abscesses, and no abnormalities of the lymphoid follicles, which play an important rôle in bacillary dysentery,[3] were observed. Seventy-one cases (39 per cent) of this series were of crypt abscess type, and 18 cases were fatal.

In 3 necropsies vasculitis and crypt abscesses were observed together.

Focal granulomas were present in 11 of the 71 crypt abscess cases, either in the colon or regional lymph nodes. They resembled the tubercles of sarcoid, and frequently contained giant cells. Sometimes a granuloma originated from the site of a crypt abscess, but granulomas did not accompany vascular lesions. Seven of these cases were clinically and pathologically indistinguishable otherwise from ulcerative colitis, and the granulomas were considered to be incidental secondary manifestations, such as are met with in some examples of carcinoma of the stomach or breast. The remaining 4 cases were controversial, since diagnoses of cicatrizing enteritis (regional ileitis) extending to the colon might be entertained.[88] All of them had involvement of the small intestine, affecting up to 68 cm. of ileum. Concomitantly, the entire length of the colon showed changes grossly and microscopically compatible with ulcerative colitis. Probably both diseases were present.

An indeterminate microscopic appearance was found in 90 cases (50 per cent). The pathologic changes either were so extensive or of such

long duration that it was impossible to decide how they developed. Thirty of these cases were fatal. Lymphoid follicles with germinal centers were prominent in the submucosa of 20 indeterminate, 19 crypt abscess, and 3 vasculitis cases. They were considered to result from long-continued ulceration and secondary bacterial infection. Pseudopolyps were present in 28 of the indeterminate cases (Fig. 10). By means of multiple sections and special attention to a search for early lesions, it would probably be possible in future studies to reduce the number of indeterminate cases. The indeterminate group may include lesions of types as yet undescribed, as well as vasculitis and crypt abscess cases.

Intestinal changes secondary to ulcerative colitis comprised, from the lumen outward, mucosal regeneration, hypertrophy of the muscularis mucosae, submucosal fibrosis and fatty infiltration, muscular hypertrophy, and subserosal fibrosis (Fig. 11). Epithelium at first covered the healing surface to a variable extent with a single layer of cuboidal cells, which later formed primitive glands but rarely reproduced the orderly, well differentiated appearance of normal mucosa. The muscularis mucosae often became markedly thickened. The submucosa was densely scarred and infiltrated with fat. Muscular hypertrophy, particularly of the circular layer, was the rule. Mild fibrosis was found in the subserosal connective tissue. The regional lymph nodes, at first edematous and hyperplastic, eventually developed fibrous thickening of their capsules.

Complications

One of the distressing features of ulcerative colitis is the number and variety of complications, attributable to inflammation, lowered resistance, malnutrition, and unknown processes. The incidence of complications in this series is given in Table II and compared with clinical series previously reported.[6,7,39-41] Perforation of the colon or ileum, either spontaneously or postoperatively, is the most lethal. Either a local abscess or generalized peritonitis may result, and the latter is more common. Other acute events are ileal intussusception and perirectal abscesses, which rupture and form fistulas in ano. Later complications following scarring of the colon include stricture or even obliteration of a portion of the lumen. Previous ileostomy encourages cicatricial contraction, but is not a prerequisite.

Carcinoma arising in ulcerative colitis has attracted considerable and deserved attention.[42-44] Nine cases (5 per cent) of this series developed carcinoma of the colon, frequently of high-grade malignancy, and in 2 cases carcinoma arose in multiple sites. The average age of these patients was 41 years, with duration of ulcerative colitis from 3 to 13 years.

Carcinoma develops in ulcerative colitis by two processes. First, neoplastic mucosal polyps are present in patients with ulcerative colitis to the same degree as in the general population, and have the same tendency to become malignant, accelerated, perhaps, by chronic inflammation. Second, regeneration of mucosal epithelium continues despite repeated ulcerations, and eventually foci of precancerous epithelial hyperplasia develop in the unfavorable environment. Such areas were observed in 4 cases in this series, with an average duration of 10 years of ulcerative colitis. Inflammatory pseudopolyps, because of their structure, are considered least likely to undergo neoplastic transformation, and no convincing case of this has been found.

TABLE II

Complications of Ulcerative Colitis

	Present series 180 cases	Collected from literature 2029 cases
Intestinal		
Perforation	15 (8%)	41 (2.0%)
Ileum 5		
Colon 10		
Peritonitis	27 (15%)	
Abscess or fistula	11 (6%)	95 (4.7%)
Stricture	3 (2%)	134 (6.6%)
Carcinoma	9 (5%)	37 (1.8%)
Multiple primary sites	2 (1%)	
Nutritional		
Emaciation	12 (7%)	
Anasarca	5 (3%)	26 (1.3%)
Fatty liver	33 (18%)	
Cause undetermined		
Thrombosis	12 (7%)	26 (1.3%)
Pancreatic dystrophy	45 (25%)	
Liver necrosis	6 (3%)	
Liver cirrhosis	3 (2%)	
Nephritis, all types	17 (9%)	35 (1.7%)
Arthritis	5 (3%)	93 (4.6%)
Skin disease	16 (9%)	77 (3.8%)
Endocarditis	3 (2%)	17 (0.8%)
Myocarditis	3 (2%)	
Amyloidosis	3 (2%)	
Adrenal necrosis	1	

Apart from the intestinal tract, thrombosis was the most common, relatively acute complication. It occurred in two-fifths of the autopsied cases, most frequently in the iliac veins, but occasionally elsewhere, as in the jugular veins, aorta, and mesenteric arteries (Fig. 12). Mural cardiac thrombi were present in 3 cases. Generalized thrombophlebitis and arterial thromboses in ulcerative colitis are serious rare complications.[45,46]

A type of pancreatic dystrophy, recently investigated,[47] was found in three-fourths of the 60 autopsies. It consists of focal dilatation of terminal ducts and related acini, which are filled with plugs of eosinophil

secretion, at times layered. Some acinar cells in affected areas show metaplastic eosinophilic cytoplasm (Fig. 13). The appearance strongly suggests local stagnation of exocrine secretion, not due to mechanical obstruction or other abnormality of larger ducts. Cause of the dystrophy is unknown. One suggestion is secretin deficiency.[47] In a control series of 100 autopsies selected to include diseases thought most likely to show this condition, 41 per cent had the same lesions. The pancreatic dystrophy thus appears to be nonspecific.

Malnutrition is a usual sequel to ulcerative colitis.[48] Emaciation results in many cases. Associated anemia[49] and lowered plasma protein are frequent,[50] and anasarca may ensue. Fatty liver, probably also a by-product of malnutrition,[51] was found in 33 of 60 autopsies. Focal hepatic necrosis was observed in 6 cases (Fig. 14). Three other patients had hepatic cirrhosis at autopsy.[52] In two the condition was considered to be toxic cirrhosis, with perilobular chronic inflammation, fibrosis, and disorganization of normal liver structure (Fig. 15). The third showed an early stage of cirrhosis arising in a fatty liver.

Renal disease, usually pyelonephritis, was found in almost 10 per cent of this series. The frequency of skin diseases, particularly focal necrosis, with ulcerative colitis is already well known.[53,54] Arthritis is often present, and the Marie-Strümpell type may be encountered more frequently than might be expected.[55] Toxic myocarditis and amyloidosis[56] were each present in 3 cases. Some rarer complications reported, such as combined systemic disease,[57] hypertrophic osteo-arthropathy,[17,58] infantilism,[59,60] and calcinosis[61] were not encountered.

TABLE III

Coincidental Diseases in 60 Autopsied Cases

Cholelithiasis	8
Diabetes mellitus	3
Obesity	3
Peptic ulcer	3
Rheumatic heart disease	3
Myocardial infarct	2
Carcinoma of breast, cervix, gallbladder, and kidney	1 each

Coincidental diseases found with ulcerative colitis are listed in Table III. Four patients had had surgical operations or trauma which might have been factors in precipitating ulcerative colitis.

CAUSES OF DEATH

The immediate causes of death in the 60 cases upon which autopsies were performed are tabulated in Table IV, with 41 autopsy reports from other sources.[5,7,10,11,17,46,56,59] In the present series, peritonitis was re-

sponsible for 21 deaths; pulmonary diseases exclusive of embolism for 8; and renal failure and sepsis, for 7 each. The 5 cases of pulmonary

TABLE IV
Causes of Death

	60 autopsied cases, present series	41 autopsied cases, from literature	Total 101 cases
Peritonitis (postoperative, 12)	21	21	42
Emaciation	3	12	15
Bronchopneumonia	2	10	12
Sepsis or toxemia	7	3	10
Renal insufficiency	7	3	10
Thrombosis	2	5	7
Pulmonary embolus	5	1	6
Hemorrhage or anemia	4	0	4
Ileus	2	1	3
Cardiac insufficiency	0	3	3
Lung abscess	2	0	2
Pulmonary edema	2	0	2
Pulmonary tuberculosis	1	1	2
Hepatic insufficiency	1	1	2
Empyema	1	0	1
Undetermined	5	1	6
Multiple causes	5	24	29

embolus correspond to the expected incidence.[62] Gastro-intestinal or intraperitoneal hemorrhage, massive[63] or insidious, caused 4 deaths.

ETIOLOGY AND PATHOGENESIS

Continued interest in specific bacterial agents in ulcerative colitis has produced many interesting studies but no convincing results. Bargen's diplostreptococcus[1] and the *Bacterium necrophorum* of Dragstedt, Dack, and Kirsner[64] are at present regarded as secondary invaders.[65-67] The possible rôle of viruses has not yet been exhaustively investigated. Felsen's thesis[3] that both cicatrizing enteritis and ulcerative colitis result from bacillary dysentery lacks sufficient bacteriologic and morphologic proof, and was not supported by the microscopic findings in the present series. However, dysentery may be one of several trigger mechanisms setting off an ulcerative process.[67]

Psychogenic factors as a background for ulcerative colitis have received general attention.[5,68-70] Personality disorders such as excessive dependency, immature emotional reactions, and over-conscientiousness have been emphasized, as well as prolonged emotional tension. Psychoses are rarely associated with colitis, but mental abnormalities of less severe type are encountered frequently enough to be noticed by many physicians, and ulcerative colitis is sometimes a psychosomatic disease.[71] It is the mechanism by which emotional stress leads to ulceration which is of particular interest to the pathologist. The ingenious experiments

of Lium and Porter[72,73] indicate that spasm of the colon can produce ulceration. But in our series little support was found for their idea that the ulcers lie in relation to taenial muscle bands. Hypertrophic changes predominate in the circular muscle and muscularis mucosae, not in the taeniae. Spasticity of the colon is a common condition not associated with ulceration, and in ulcerative colitis muscular hypertrophy results from the spastic contraction secondary to mucosal ulceration. The muscular thickening is an effect and not the cause of ulceration.

The chief manifestations of ulcerative colitis are seen in and just beneath the mucosa. The gross appearance of the colon often suggests the presence of a destructive substance in the fecal stream,[74] and this impression is strengthened by the improvement in symptoms and in sigmoidoscopic appearance of the mucosa following ileostomy.[75,76] Analysis of feces from patients with ulcerative colitis has shown abnormal quantities of proteolytic enzymes in some cases.[77,78] It is possible that intestinal hypermotility has introduced gastric and small-intestinal enzymes into the colon,[68,79] although increased peristalsis is present in a minority of cases of ulcerative colitis.[80] Evidence that bile or pancreatic juice is the responsible agent has not been forthcoming. Patients with cholecystocolic fistulas only rarely suffer from diarrhea.[81] Animal experiments along similar lines have produced negative results, and patients with ulcerative colitis excrete less trypsin from ileostomy openings than patients with carcinoma.[82]

Colonic mucosa has excretory as well as absorptive functions, and the formation of crypt abscesses suggests that some substance is being secreted or excreted into the affected crypts.[74] No abscesses or other lesions are found in the connective tissue or vessels, which implies that the substance does not become a leukotactic irritant until altered by a change of pH or otherwise after its discharge into the crypt lumina. Release of an enzyme of proteolytic type in the bases of the crypts is an attractive idea which remains unproved. It is of interest that similar lesions occur in the development of chronic gastritis.[83] Three of our cases had analogous abnormal mucosal crypts in the esophagus, stomach, or small intestine, or in combinations of these organs. The crypts contained laminated eosinophilic mucoid material which reacted consistently to several different stains. This material was not regularly leukotactic, and its relationship to ulcerative colitis is uncertain.

Discussion

Differential diagnosis of colitis of specific etiology has been well discussed elsewhere.[1,2] Chronic bacillary dysentery and ulcerative colitis have given rise to most controversy. Authorities are about equally

divided as to whether they are pathologically distinct[1,7,8,66,67,74] or indistinguishable.[3,6,24,84-86] Our experience inclines us to believe that the histologic characteristics differ. Ash and Spitz[87] described subepithelial mucosal cysts in chronic dysentery, and, in the acute stage, superficial mucosal inflammation and diphtheritic membrane formation. These changes are not observed in ulcerative colitis, although the early stages of the indeterminate group were, of course, not made out. In most discussions of the differentiation of chronic dysentery and ulcerative colitis, insufficient evidence is presented to allow one to draw definite conclusions, and the subject remains mainly a collection of opinions unsupported by published observations. No claim of specificity is advanced for the early lesions described in our cases of ulcerative colitis. Vasculitis may accompany bacillary dysentery, mesenteric thrombosis, or volvulus. Crypt abscesses have been found in control cases of amebic dysentery, lymphopathia venereum, and in one case of generalized vitamin deficiency. All these conditions may at times have similar lesions leading to ulceration.

Cicatrizing enteritis is occasionally very difficult to differentiate from ulcerative colitis.[26,88] This is partly due to inadequate knowledge of the etiology of both diseases, and partly to their coexistence in occasional patients.[89] The 4 cases in this series in which both diagnoses were made emphasize that, since the two conditions may affect the same patient, final distinctions are not always necessary. This does not, however, necessarily justify the conclusion that both diseases are part of a single entity. In fact, they usually differ in distribution and in gross and microscopic appearances, as well as clinically. Vasculitis and crypt abscesses have not been observed by us in cicatrizing enteritis. Among 20 recent cases of cicatrizing enteritis, small groups of macrophages and eosinophilic leukocytes were found in the mucosal crypts of 6. Conversely, the extensive granuloma formation of cicatrizing enteritis[88] does not characterize ulcerative colitis. The scattered foci resembling those of sarcoid occasionally found in ulcerative colitis are similar to those which at times accompany other intestinal diseases such as carcinoma, lymphoma, and diverticulitis. However, the problems of pathogenesis involved are still far from final solution.

Vessels affected by vasculitis usually were confined to the colon. That the lesions are produced by a circulating substance, analogous to the development of glomerulonephritis or periarteritis nodosa, seems reasonable. What causes the colon to be predisposed is unknown.

Crypt abscesses were the most common precursors of ulceration. Preceding hyperemic and edematous mucosal stages have been described

during proctoscopy. The unaltered histologic appearance of all but localized portions of the mucosa near the bases of certain crypts, where the epithelium is flattened by polymorphonuclear leukocytes in the lumen, makes it seem unlikely that absorption of water from the feces has produced a destructive concentration of some solute. Presence of early lesions in a uniform location within the crypts, even though scattered along the course of the intestine, suggests instead that the irritant is being released by the mucosa. The possible rôle of such agents as proteolytic enzymes in ulcerative colitis already has aroused much clinical interest, but their origin from the colonic mucosa has not been considered previously and now requires further investigation.

The importance of psychosomatic influences in the genesis of ulcerative colitis cannot be disregarded. It is already known that fear and other emotional stresses can provoke the release of potent circulating chemical agents, including substances with proteolytic properties,[90,91] and some authors have thought that ulcerative colitis resembles the effect of a circulating toxin upon the colon.[8,92] Any connection of proteolytic enzymes with the pathogenesis of ulcerative colitis remains to be established.

Summary

A series of 120 surgical and 60 autopsied cases of ulcerative colitis, a nonspecific chronic intestinal disease particularly affecting young adults, was analyzed pathologically. The sigmoid and descending colon were most frequently ulcerated (83 per cent), and the cecum least often (61 per cent). Investigation of ulcer formation indicated that local vasculitis was responsible in 19 cases (11 per cent), and mucosal crypt abscesses in 71 (39 per cent). These types of colitis also differed significantly in sex and age incidences, gross appearance, and mortality. Ninety cases (50 per cent) were of indeterminate origin. Peritonitis, fatty liver, and a pancreatic dystrophy were the most frequent of many complications observed. As to the etiology of lesions leading to ulceration various possibilities have been considered, but the importance of particular factors remains unestablished.

The greater part of the surgical material was from the Surgical Service of the Lahey Clinic, and other surgical cases were from the practices of Drs. Thomas J. Anglem, James M. Bonnar, Jr., Howard M. Clute, Daniel F. Jones, Halsey B. Loder, Charles C. Lund, Leland S. McKittrick, and Irving J. Walker.

REFERENCES

1. Bargen, J. A. The Modern Management of Colitis. Charles C Thomas, Baltimore, 1943, 322 pp.
2. Monaghan, J. F. Ulcerative Colitis. In: Bockus, H. L. Gastroenterology. W. B. Saunders Co., Philadelphia & London, 1944, **2**, 549–614.

3. Felsen, J. Bacillary Dysentery, Colitis and Enteritis. W. B. Saunders Co., Philadelphia and London, 1945, 618 pp.
4. Palmer, W. L. Chronic ulcerative colitis. *Gastroenterology*, 1948, **10**, 767–781.
5. Kirsner, J. B., Palmer, W. L., Maimon, S. N., and Ricketts, W. E. Clinical course of chronic nonspecific ulcerative colitis. *J. A. M. A.*, 1948, **137**, 922–928.
6. Hurst, A. F. Ulcerative colitis. *Guy's Hosp. Rep.*, 1935, **85**, 317–355.
7. Monroe, C. W. Chronic nonspecific ulcerative colitis; review of 138 cases. *Surgery*, 1937, **2**, 575–580.
8. Hern, J. R. B. Ulcerative colitis. *Guy's Hosp. Rep.*, 1931, **81**, 322–373.
9. Hardy, T. L., and Bulmer, E. Ulcerative colitis; a survey of 95 cases. *Brit. M. J.*, 1933, **2**, 812–815.
10. Cabot case 28252. *New England J. Med.*, 1942, **226**, 997–1000.
11. Hart, J. A. Ulcerative colitis with perforation in a newborn; report of a case. *Texas State J. Med.*, 1946–47, **42**, 286–287.
12. Jackman, R. J., Bargen, J. A., and Helmholz, H. F. Life histories of 95 children with chronic ulcerative colitis; a statistical study based on comparison with a whole group of 871 patients. *Am. J. Dis. Child.*, 1940, **59**, 459–467.
13. Litvak, A. M., and Levy, H. Nonspecific ulcerative colitis in childhood; case report and review of the literature. *Arch. Pediat.*, 1944, **61**, 293–299.
14. Brust, J. C. M., and Bargen, J. A. Chronic ulcerative colitis among elderly persons. *Minnesota Med.*, 1935, **18**, 583–585.
15. Hawkins, H. P. On the natural history of ulcerative colitis and its bearing on treatment. *Brit. M. J.*, 1909, **1**, 765–770.
16. Cullinan, E. R. Ulcerative colitis; clinical aspects. *Brit. M. J.*, 1938, **2**, 1351–1356.
17. Schlicke, C. P., and Bargen, J. A. Fulminating ulcerative colitis; a critical analysis of 20 cases. *Minnesota Med.*, 1940, **23**, 348–352.
18. Bargen, J. A., Jackman, R. J., and Kerr, J. G. Studies on the life histories of patients with chronic ulcerative colitis (thrombo-ulcerative colitis), with some suggestions for treatment. *Ann. Int. Med.*, 1938, **12**, 339–352.
19. Crohn, B. B., Garlock, J. H., and Yarnis, H. Right-sided (regional) colitis. *J. A. M. A.*, 1947, **134**, 334–338.
20. Banks, B. M., and Bargen, J. A. Relapses in chronic ulcerative colitis; causes and prevention. *Arch. Int. Med.*, 1934, **53**, 131–139.
21. Crohn, B. B., and Rosenak, B. D. A follow-up of ulcerative colitis (nonspecific). *Am. J. Digest. Dis. & Nutrition*, 1935, **2**, 343–346.
22. Willard, J. H., Pessel, J. F., Hundley, J. W., and Bockus, H. L. Prognosis of ulcerative colitis. *J. A. M. A.*, 1938, **111**, 2078–2084.
23. Crohn, B. B., and Berg, A. A. Right-sided (regional) colitis. *J. A. M. A.*, 1938, **110**, 32–38.
24. Rudner, H. G. Chronic ulcerative colitis. *South. M. J.*, 1935, **28**, 429–434.
25. Kiefer, E. D. Personal communication.
26. Crohn, B. B., and Rosenak, B. D. A combined form of ileitis and colitis. *J. A. M. A.*, 1936, **106**, 1–7.
27. Gray, H. Anatomy of the Human Body. (W. H. Lewis, ed.) Lea & Febiger, Philadelphia, 1942, p. 1196.
28. Moses, W. R. Meckel's diverticulum; report of two unusual cases. *New England J. Med.*, 1947, **237**, 118–122.
29. Moore, R. A. A Textbook of Pathology. W. B. Saunders Co., Philadelphia & London, 1945, p. 312.
30. Swinton, N. W., and Haug, A. D. The frequency of precancerous lesions in the rectum and colon. *Lahey Clin. Bull.*, 1947, **5**, 84–88.

31. Coffey, R. J., and Bargen, J. A. Intestinal polyps: pathogenesis and relation to malignancy. *Surg., Gynec. & Obst.*, 1939, **69**, 136–145.
32. Swinton, N. W., and Warren, S. Polyps of the colon and rectum and their relation to malignancy. *J. A. M. A.*, 1939, **113**, 1927–1933.
33. Helwig, E. B. The evolution of adenomas of the large intestine and their relation to carcinoma. *Surg., Gynec. & Obst.*, 1947, **84**, 36–49.
34. Lium, R., and Porter, J. E. Observations on the etiology of ulcerative colitis. III. The distribution of lesions and its possible significance. *Am. J. Path.*, 1939, **15**, 73–77.
35. Lium, R. Observations on the etiology of ulcerative colitis. IV. The rectometrogram and the rectal reactions of eight normal subjects and one patient with ulcerative colitis before and after spinal anesthesia. A preliminary report. *Am. J. M. Sc.*, 1939, **197**, 841–847.
36. Bargen, J. A., and Weber, H. M. Clinical and roentgenologic aspects of chronic ulcerative colitis. *Radiology*, 1931, **17**, 1153–1157.
37. Buie, L. A. Chronic ulcerative colitis. *J. A. M. A.*, 1926, **87**, 1271–1274.
38. Warren, S., and Sommers, S. C. Cicatrizing enteritis (regional ileitis) as a pathologic entity. Analysis of one hundred and twenty cases. *Am. J. Path.*, 1948, **24**, 475–501.
39. Jankelson, I. R., McClure, C. W., and Sweetsir, F. N. Chronic ulcerative colitis II: complications outside the digestive tract. *Rev. Gastroenterol.*, 1942, **9**, 99–104.
40. Ricketts, W. E., and Palmer, W. L. Complications of chronic nonspecific ulcerative colitis. *Gastroenterology*, 1946, **7**, 55–66.
41. Bargen, J. A. Chronic Ulcerative Colitis. In: Piersol, G. M. (ed.) The Cyclopedia of Medicine, Surgery and Specialties. F. A. Davis Co., Philadelphia, 1946, **4**, 382–398.
42. Sauer, W. G., and Bargen, J. A. Chronic ulcerative colitis followed by carcinoma: report of 26 cases. *Proc. Staff Meet., Mayo Clin.*, 1944, **19**, 311–315.
43. Lynn, D. H. The relationship of chronic lesions to carcinoma of the colon—chronic ulcerative colitis; collective review. *Surg., Gynec. & Obst.* (Abstract Sect.), 1945, **81**, 269–276.
44. Cattell, R. B., and Boehme, E. J. The importance of malignant degeneration as a complication of chronic ulcerative colitis. *Gastroenterology*, 1947, **8**, 695–710.
45. Bargen, J. A., and Barker, N. W. Extensive arterial and venous thrombosis complicating chronic ulcerative colitis. *Arch. Int. Med.*, 1936, **58**, 17–31.
46. Martin, M. E. Arterial thrombosis associated with chronic ulcerative colitis. *Am. J. Digest. Dis.*, 1945, **12**, 85–88.
47. Baggenstoss, A. H., Power, M. H., and Grindlay, J. H. The relationship of fibrocystic disease of the pancreas to a deficiency of secretin. (Abstract.) *Am. J. Path.*, 1948, **24**, 692–694.
48. Bercovitz, Z., and Page, R. C. Metabolic and vitamin studies in chronic ulcerative colitis. *Ann. Int. Med.*, 1944, **20**, 239–254.
49. Pollard, H. M., Block, M., and Bachrach, W. H. Causes and management of anemia associated with chronic ulcerative colitis. *J. A. M. A.*, 1947, **134**, 341–346.
50. Ross, J. R., and Swarts, J. M. Hepatic dysfunction and cirrhosis in chronic ulcerative colitis. *Gastroenterology*, 1948, **10**, 81–95.
51. Moschcowitz, E. Laennec cirrhosis: Its histogenesis, with special reference to the role of angiogenesis. *Arch. Path.*, 1948, **45**, 187–215.
52. Tumen, H. J., Monaghan, J. F., and Jobb, E. Hepatic cirrhosis as a complication of chronic ulcerative colitis. *Ann. Int. Med.*, 1947, **26**, 542–553.

53. Mintzer, I. J. Pyoderma gangraenosum, onychogryphosis and onycholysis with ulcerative colitis. *Arch. Dermat. & Syph.*, 1939, **40**, 541–543.
54. Felsen, J. Multiple necrotizing skin lesions in chronic ulcerative colitis. *New York State J. Med.*, 1941, **41**, 2228–2231.
55. Hare, H. F. Personal communication.
56. Cabot case 33021. *New England J. Med.*, 1947, **236**, 75–78.
57. Suh, T. H., and Merritt, H. H. Combined system disease without obvious evidence of pernicious (macrocytic) anemia. *Am. J. M. Sc.*, 1938, **196**, 57–66.
58. Mendlowitz, M. Clubbing and hypertrophic osteoarthropathy. *Medicine*, 1942, **21**, 269–306.
59. Davidson, S. Infantilism in ulcerative colitis. *Arch. Int. Med.*, 1939, **64**, 1187–1196.
60. Ricketts, W. E., Benditt, E. P., and Palmer, W. L. Chronic ulcerative colitis with infantilism and carcinoma of the colon. *Gastroenterology*, 1945, **5**, 272–280.
61. Rasmussen, H. Transient calcinosis, accompanying ulcerative colitis. *Acta med. Scandinav.*, 1939, **101**, 491–495.
62. Dehlinger, K., and Riemenschneider, P. Pulmonary embolism. Analysis of 74 autopsy cases since 1941. *New England J. Med.*, 1949, **240**, 497–501.
63. Sealy, W. B., and Brown, P. W. Massive hemorrhage complicating ulcerative colitis. *Proc. Staff Meet., Mayo Clin.*, 1940, **15**, 497–499.
64. Dragstedt, L. R., Dack, G. M., and Kirsner, J. B. Chronic ulcerative colitis; a summary of evidence implicating *Bacterium necrophorum* as an etiologic agent. *Ann. Surg.*, 1941, **114**, 653–662.
65. Bassler, A. The bacteriology of ulcerative colitis. *M. J. & Rec.*, 1933, **138**, 472–478.
66. Willard, J. H. Ulcerative colitis. A review. *Internat. Clinics*, 1939, **4**, 281–298.
67. Ginsberg, R. S., and Ivy, A. C. Etiology of ulcerative colitis; analytical review of the literature. *Gastroenterology*, 1946, **7**, 67–90.
68. Sullivan, A. J. Psychogenic factors in ulcerative colitis. *Am. J. Digest. Dis. & Nutrition*, 1935, **2**, 651–656.
69. Wittkower, E. Ulcerative colitis: personality studies. *Brit. M. J.*, 1938, **2**, 1356–1360.
70. Daniels, G. E. Psychiatric aspects of ulcerative colitis. *New England J. Med.*, 1942, **226**, 178–184.
71. Cobb, S. Borderlands of Psychiatry. Harvard University Press, Cambridge, 1946, p. 156.
72. Lium, R., and Porter, J. Etiology of ulcerative colitis. I. The preparation, care and secretions of colonic explants in dogs. *Arch. Int. Med.*, 1939, **63**, 201–209.
73. Lium, R. Etiology of ulcerative colitis. II. Effect of induced muscular spasm on colonic explants in dogs, with comment on relation of muscular spasm to ulcerative colitis. *Arch. Int. Med.*, 1939, **63**, 210–225.
74. Manson-Bahr, P. The Dysenteric Disorders. Williams & Wilkins Co., Baltimore, 1943, pp. 436 and 438.
75. Jones, T. E. The surgical treatment of ulcerative colitis. *J. A. M. A.*, 1938, **111**, 2076–2078.
76. Cattell, R. B. The surgical treatment of ulcerative colitis. *Gastroenterology*, 1948, **10**, 63–66.
77. Meyer, K., Gellhorn, A., Prudden, J. F., Lehman, W. L., and Steinberg, A. Lysozyme in chronic ulcerative colitis. *Proc. Soc. Exper. Biol. & Med.*, 1947, **65**, 221–222.
78. Warren, S., and Sommers, S. C. (In preparation.)
79. Ehrlich, R. Pathogenesis and treatment of ulcerative colitis with extract of hog stomach. *Am. J. Digest. Dis.*, 1947, **14**, 294–297.

80. Mackie, T. T. The medical management of chronic ulcerative colitis. *J. A. M. A.*, 1938, **111**, 2071–2076.
81. Wakefield, E. G., Vickers, P. M., and Walters, W. Cholecystoenteric fistulas. *Surgery*, 1939, **5**, 674–677.
82. Ivy, J. H., and Clarke, B. G. Is bile and pancreatic juice a factor in the genesis of ulcerative colitis? An experimental study. *Gastroenterology*, 1945, **5**, 416–417.
83. Collins, V. P. The peptic ulcer and chronic gastritis. *Ann. Surg.*, 1943, **118**, 1005–1014.
84. Mackie, T. T. Ulcerative colitis due to chronic infection with Flexner-Y bacillus; report of case with cure by autogenous vaccine. *J. A. M. A.*, 1932, **98**, 1706–1710.
85. Harrison, P. E., and Banvard, J. Coproantibody excretion during enteric infections. *Science*, 1947, **106**, 188–189.
86. Harrison, P. E. Significance of coproantibody excretion during enteric infection. *J. Lab. & Clin. Med.*, 1947, **32**, 1409.
87. Ash, J. E., and Spitz, S. Pathology of Tropical Diseases. W. B. Saunders Co., Philadelphia & London, 1945, p. 78.
88. Epstein, H. J., Lipshutz, B., and Steinbach, M. Regional colitis involving the descending colon and sigmoid. *Am. J. Digest. Dis.*, 1947, **14**, 13–16.
89. Kiefer, E. D. The diagnosis of regional ileitis (chronic idiopathic ulcerative enteritis). *S. Clin. North America*, 1943, **23**, 862–872.
90. Macfarlane, R. G., and Pilling, J. Observations on fibrinolysis; plasminogen, plasmin, and antiplasmin content of human blood. *Lancet*, 1946, **2**, 562–565.
91. Macfarlane, R. G., and Biggs, R. Observations on fibrinolysis; spontaneous activity associated with surgical operations, trauma, etc. *Lancet*, 1946, **2**, 862–864.
92. Riely, L. A. Chronic ulcerative colitis. *South. M. J.*, 1935, 28, 370–376.

DESCRIPTION OF PLATES

PLATE 102

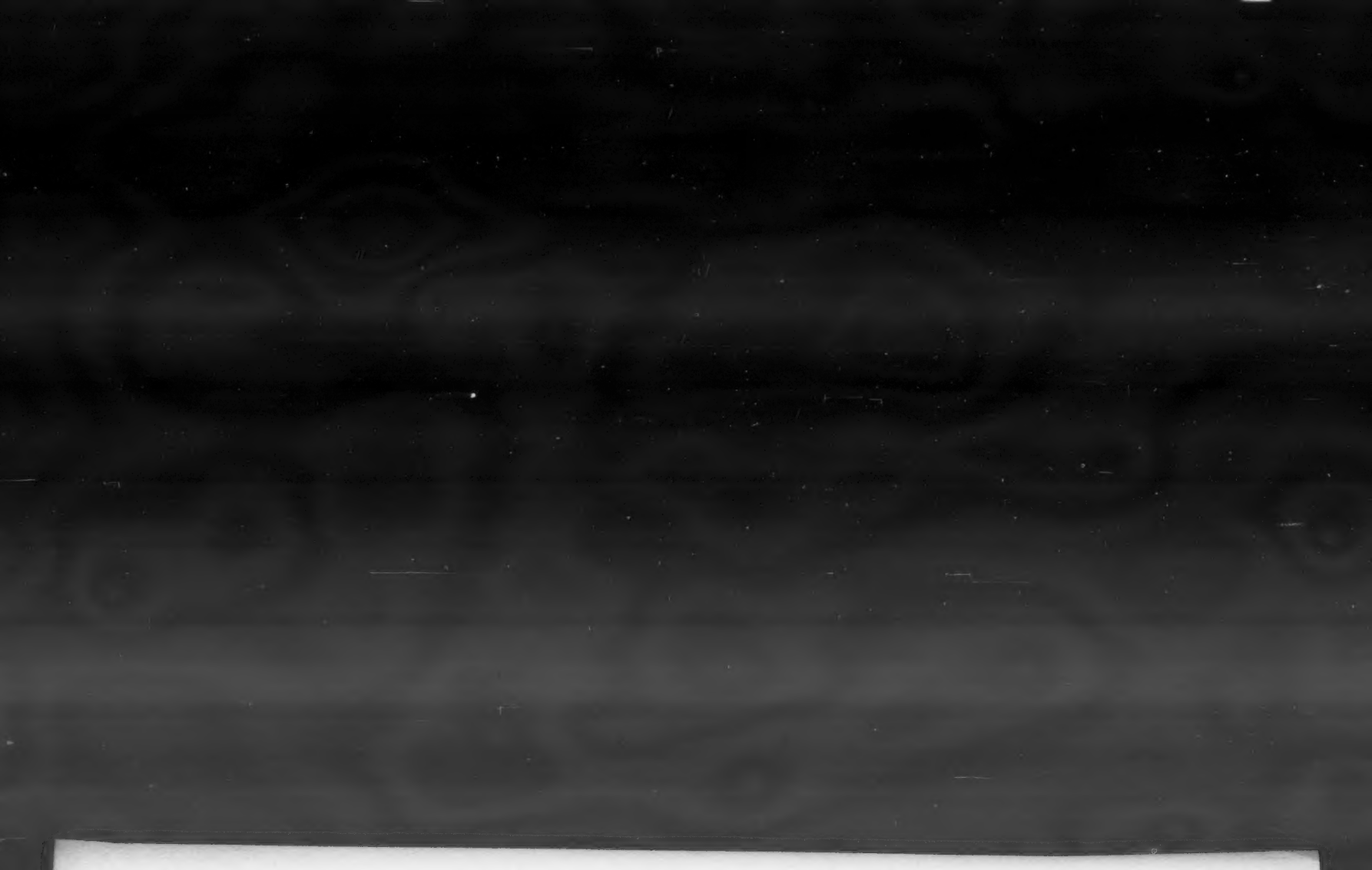
FIG. 1. Mucosal surface of colon, showing localized severe ulcerative colitis in a man, 28 years old. Dark raised islands of persistent or regenerating mucosa are seen in the light-colored region of ulceration. Histologically, the lesions were of indeterminate type.

FIG. 2. Colonic mucosa showing network of ulcers with pseudopolyps between them, from a case of ulcerative colitis in a woman, 26 years old. The specimen had been fixed in formalin with resulting shrinkage of the pseudopolyps, which in the fresh state concealed the ulcers. Microscopically, crypt abscesses were present.

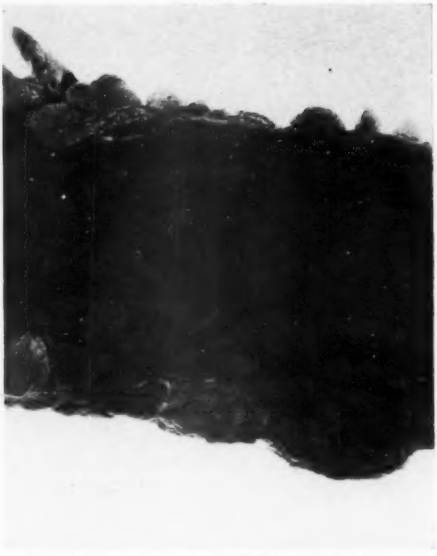
FIG. 3. Early vasculitis in the submucosa of the colon. Of note is the marked vascular necrosis with relatively normal appearance of the overlying mucosa. Grossly, extensive ulceration was present. Eosin and methylene blue stain. × 150.

FIG. 4. Vascular necrosis and inflammation in a vessel, larger than that shown in Figure 3, in the wall of the colon. Thrombosis had not yet occurred. Eosin and methylene blue stain. × 150.

FIG. 5. Thrombosis of a submucosal vein in another case which showed vasculitis with ulcerative colitis. Eosin and methylene blue stain. × 125.

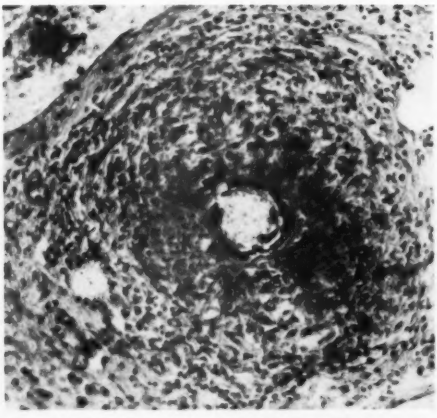


1

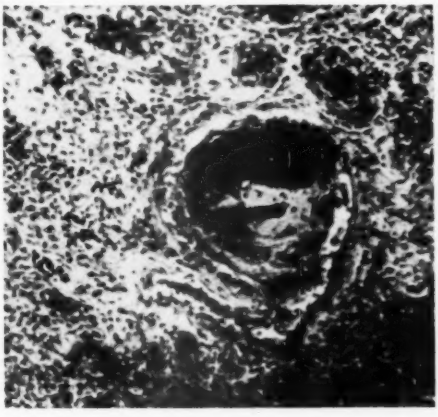


2

3



4



5

PLATE 103

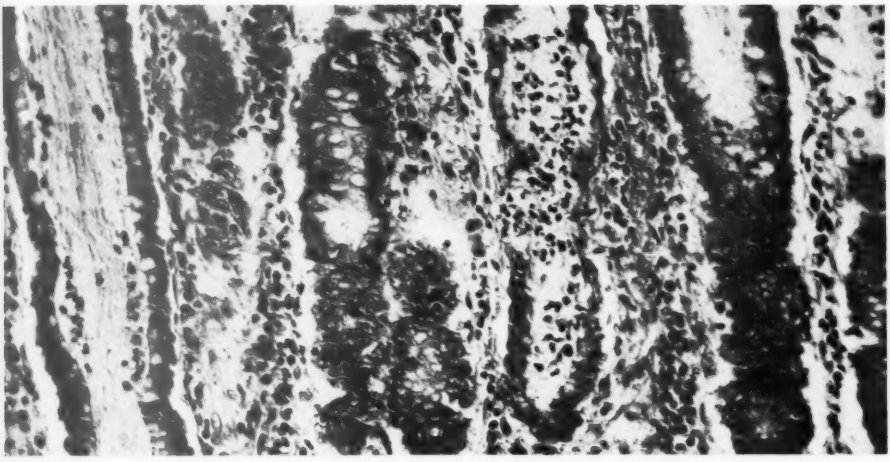
FIG. 6. Early stage of crypt abscess formation in ulcerative colitis. Polymorphonuclear leukocytes are found in localized regions near the bases of some crypts. Stringy eosinophilic mucoid secretion is present. The epithelium is not definitely abnormal except where it is compressed, and the lamina propria appears negative. Eosin and methylene blue stain. × 250.

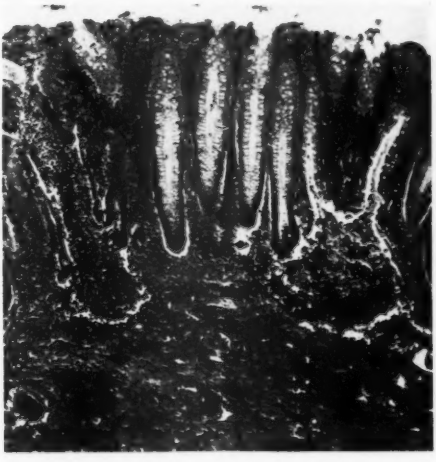
FIG. 7. Well developed crypt abscess compressing and rupturing through the base of a crypt. Eosin and methylene blue stain. × 50.

FIG. 8. Two ruptured crypt abscesses. The mucosa between would have sloughed with formation of an ulcer, although it still appears normal. Eosin and methylene blue stain. × 50.

FIG. 9. Mucosal bridge in ulcerative colitis, beneath which are two ruptured crypt abscesses topped by epithelium. Circular muscle is at the bottom of the picture. Eosin and methylene blue stain. × 25.

FIG. 10. Part of a pseudopolyp. Lymphoid follicles are shown in the connective tissue, and there is epithelial regeneration. Hypertrophy of the circular muscle is also pictured. Phosphotungstic acid-hematoxylin stain. × 10.





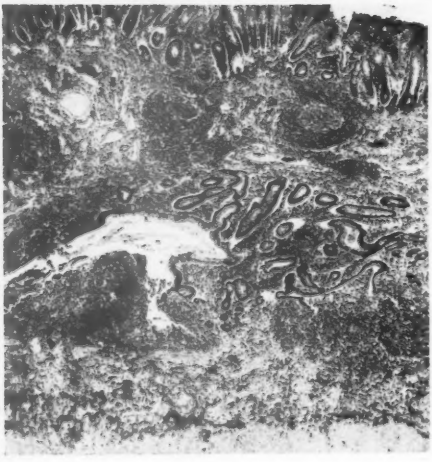


PLATE 104

FIG. 11. A late stage of ulcerative colitis of indeterminate type. From the mucosal surface downward may be seen regenerated epithelium, chronic inflammation of the upper submucosa, fatty infiltration with marked fibrosis of the lower submucosa, and hypertrophy of the circular muscle. All of the major secondary intestinal changes are present except hypertrophy of the muscularis mucosae. Phosphotungstic acid-hematoxylin stain. × 32.

FIG. 12. Organizing thrombus in the inferior mesenteric artery of a fatal case of ulcerative colitis with generalized arterial and venous thrombi. Eosin and methylene blue stain. × 10.

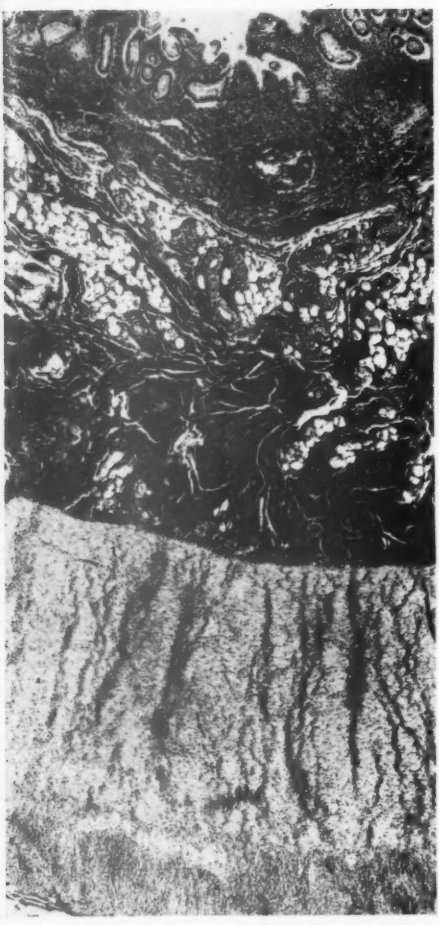
FIG. 13. Pancreatic dystrophy in ulcerative colitis, with dilatation of some terminal ducts and acini. Eosin and methylene blue stain. × 250.

FIG. 14. Focal hepatic necrosis in a fatal case of ulcerative colitis. Eosin and methylene blue stain. × 250.

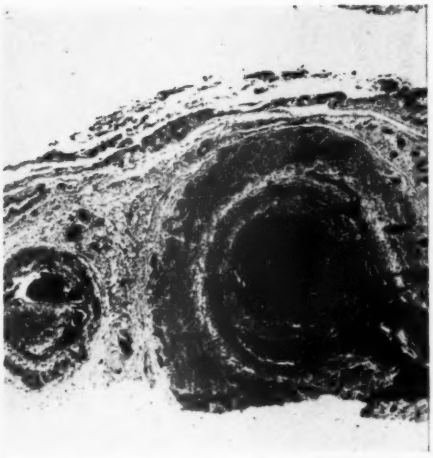
FIG. 15. Liver showing toxic cirrhosis, with marked inflammation, some fibrosis, and liver cell destruction and regeneration. The liver architecture is destroyed. Eosin and methylene blue stain. × 250.

11

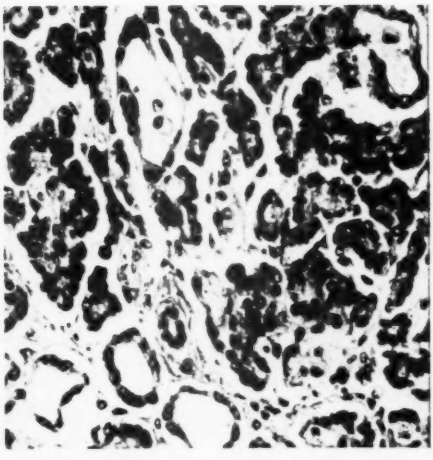
4



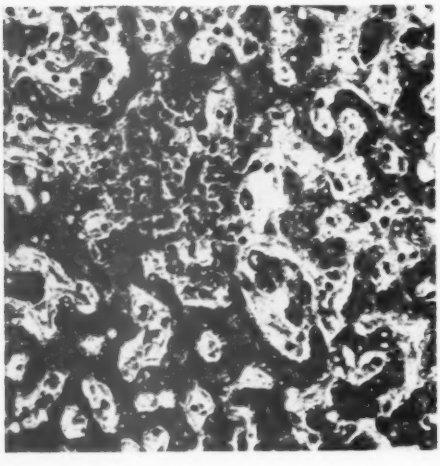
11



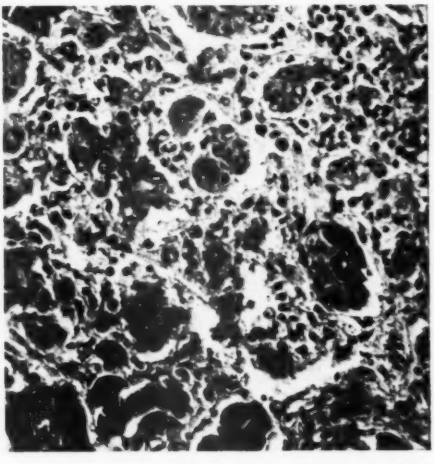
12



13



14



15

NONENCAPSULATED ADRENAL CORTICAL TISSUE IN THE PERI-ADRENAL FAT*

HERMAN C. B. DENBER, M.D.

(From the Department of Pathology, Beth Israel Hospital, New York, N.Y.)

In routine autopsy sections at this hospital, adrenal cortical tissue frequently has been observed in the surrounding fat outside the fibrous tissue capsule of the gland. The well known cortical nodules lying adjacent to the gland also at times show perforation of their capsules, with cortical tissue extending into the fat. Such extension of tissue beyond the boundaries of the organ does not fit into the accepted patterns of normal histology, yet this problem has attracted little attention from anatomists or pathologists.

A thorough review of the older literature failed to reveal any mention of the subject (Henle,[1] Huschke,[2] Kölliker,[3] Cornil and Ranvier,[4] Testut,[5] and Soulié[6]). In later years the encapsulated cortical nodules have been much discussed (Broster and Vines[7] and Gruenwald[8]). Kovács[9] described the infiltration of peri-adrenal fat by groups of cortical cells in Addison's disease, differentiating between these nonencapsulated masses and true accessory nodules. He felt that the former were the result of glandular regeneration. A similar picture was described by Huebschmann[10] and Kaiserling.[11] Sudds[12] mentioned that "nodules, islets of suprarenal cortical cells for the most part with a definite zonal arrangement and surrounded partly or entirely with fibrous tissue, were found in 64 out of 214 cases." Goldzieher[13] described nonencapsulated nodules of cortical tissue proliferating loosely into the adjacent areolar tissue in adrenal glands of cases of pemphigus vulgaris. Plaut,[14] in discussing this paper, stated that the picture of suprarenal cortex growing into the surrounding fat tissue was not a rare occurrence in his experience. He thought that this process was unrelated to any particular disease entity, and was part of the general phenomena that endocrine glands may break through their capsule and grow into the surrounding tissue (for example, pituitary body and thyroid).

Gruenwald,[8] however, believed that "outgrowth from the main gland cannot very well be assumed; it is very doubtful that normal cortical tissue should be capable of this extent of infiltrative growth." Broster and Vines[7] merely stated that "small nodules of cortical cells, usually multiple and completely separated from the main gland, though in close approximation to it, are of extreme frequency and occurred in at least 75% of normal subjects." One cannot deduce how many, if any, were

* Received for publication, August 9, 1948.

without a capsule. Goormaghtigh[15] made no comment on the subject nor did Rogoff,[16] Zwemer,[17] Maximow and Bloom,[18] Bailey,[19] or Cowdry.[20] Bennett[21] did not observe such tissue in the cat. Curiously enough, Yeakel[22] found *medullary* tissue outside of the capsule in her study of ageing rats. From the photographs, these collections appear to be encapsulated. She made no mention of having observed cortical tissue outside of the adrenal gland.

One gathers from the literature that none of the animal adrenals described showed cortical tissue in the peri-adrenal fat. Whether or not such tissue occurs only in human adrenals is problematic. In all of the animal work, mention is made of meticulous trimming of the peri-adrenal fat.

Materials and Methods

The adrenal glands of 257 adults and 36 newborn infants were examined. These were obtained from autopsies of the years 1942 to 1947. Routine paraffin sections were stained with hematoxylin and eosin. In some of the cases additional sections or ribbons were cut. Occasionally, sections stained for elastic tissue, or by the van Gieson and Bielschowsky methods were available, but there was no sudan-stained material. Attention was focused solely upon adrenal cortical tissue devoid of a capsule, indisputably lying in the peri-adrenal fat. When sections were torn, so as to make observations doubtful, the case was discarded. The frequently encountered encapsulated cortical nodules were not considered in this study. The number of sections per case varied, ranging from 1 to 28.

Observations

Of the 257 cases studied, 72 (28 per cent) showed nonencapsulated adrenal cortical tissue in the peri-adrenal fat, and 185 did not. Of the positive cases, 42 (58 per cent) were males and 30 (42 per cent), females. In the negative group there were 104 (56 per cent) males and 81 (44 per cent) females.

As Table I shows, there was a slight increase in relative incidence in the older age groups. Before 50 years of age 21.4 per cent were positive; in those 50 and older, 30.5 per cent were positive.

In 44 cases (61 per cent), a blood vessel, artery, or vein was found in, or in close proximity to, the extracapsular mass. Study of the entire surface of the gland showed that vessels tended to penetrate at certain well defined points, and the extracapsular masses usually were located at one of these points. It is probable, at least in a majority of cases, that a breakout into the fat took place at weak points in the capsule created

by the passage of a vessel, or possibly a small nerve. For some of the larger masses no blood vessels were found nearby.

TABLE I

Distribution of Positive and Negative Findings by Sex and Age in Decades

Age groups	Positive		Negative	
0–9	M–0, F–0	0	M–1, F–0	1
10–19	M–0, F–2	2	M–2, F–0	2
20–29	M–0, F–1	1	M–1, F–8	9
30–39	M–2, F–2	4	M–8, F–9	17
40–49	M–4, F–4	8	M–15, F–11	26
50–59	M–11, F–10	21	M–27, F–22	49
60–69	M–15, F–7	22	M–36, F–18	54
70–79	M–8, F–4	12	M–13, F–10	23
80–89	M–2, F–0	2	M–1, F–3	4

The masses varied in size, from a few short parallel columns of cells to relatively large nodules, one measuring 4 mm. They penetrated the fat in a fan-wise fashion, through a gap in the capsule of varying size, with tongues of tissue thrusting out between the fat cells. The shape of the mass varied from round to ovoid or oblong. Some lay entirely free in the fat, away from the capsule of the gland, while others had their longer border parallel to the capsule. The outer surface might be entirely irregular, or flat with wavy borders. The surrounding fat sometimes showed a round-celled infiltration. This usually could be explained by lesions in the adjacent viscera.

The structural arrangement within these masses of cells was very variable. In a few cases they showed the typical arrangement of the adrenal cortex. The zona glomerulosa was nearest to the capsule, with the zona fasciculata in an intermediate position, and the zona reticularis situated distally in the fat. In a great majority of the sections there was a relatively complete loss of normal architecture, often making identification of the various layers difficult. No variations from the normal cytologic picture were encountered, in spite of the apparently disordered and heterotopic growth. The capillary structure was the same as that within the gland. There was no particular hyperemia, as has been described by other authors for these extracapsular masses.[9] In the few sections available, no elastic tissue fibers were seen. That these masses were directly continuous with the intracapsular cortex was shown by continuity of the reticular fibers through the break in the capsule.

The 72 cases were reviewed to determine their zonal composition. In 25 (34.7 per cent), all of the cells were of the glomerulosa type. Cells

of glomerulosa and fasciculata types comprised 30 (41.7 per cent) of the cases. All three layers were represented in 11 (15.3 per cent) cases. In one case cells of the zona fasciculata only were present, and in another cells of fasciculata and reticularis types were identified. In 4 (0.06 per cent), cell type could not be assigned with any reasonable assurance. In 12 cases (16.7 per cent), cells of the zona reticularis were present. They were identified primarily by the presence of pigment.

The number and size of the nonencapsulated adrenal cortical masses were not related to the presence or size of encapsulated cortical nodules or to other variants in the structure of the adrenal cortex, especially hyperplastic processes.

The adrenals of 36 newborn infants were reviewed. In no case was any nonencapsulated adrenal cortical tissue found in the fat. Great care must be taken in such a study not to mistake fetal fat cells for cells of the adrenal cortex.

Adrenal medulla was neither found free in the fat nor within any of the extracapsular masses.

Discussion

The close proximity of a blood vessel in 61 per cent of the cases indicates that perforation at a weak point of the capsule represents a major means by which these masses penetrate the surrounding fat. Once having broken into the fat, they are free from the normal restraining influence of the connective tissue framework of the cortex. Consequently, the cells can grow in any fashion whatsoever. Serial sections might provide additional information as to their mode of formation. In some of the largest nodules, no adjacent blood vessel was found. It is possible that general unknown growth factors may have some relation to this bursting of anatomic boundaries. In order to substantiate Kovács'[9] contention that the extracapsular cortical masses in his cases of Addison's disease represent efforts of the gland to regenerate, one would have to study a large number of adrenal glands coming only from such cases. The material at hand is insufficient to make such an analysis.

The number of positive cases was not a matter of chance selection of microscopic sections. Positive findings were equally frequent whether there were 1, 5, 10, or 20 sections per case. For one-half of the positive cases there were only one or two sections per case.

It was thought that the presence of carcinoma might be associated with abnormal growth elsewhere. However, the positive findings in patients with malignant growths was proportional to the number with malignant tumors in the entire series. The incidence of infectious diseases

in the material studied was too low to permit conclusions. The increase of positive cases with advancing age has been pointed out. At present there is no adequate explanation for this fact. There were no examples of endocrine disturbances in this study.

It is now fairly certain that the adult adrenal cortex can be formed by differentiation of the cells of the capsule (Zwemer,[17] Wotton and Zwemer,[23] Zwemer, Wotton, and Norkus,[24] Elias[25]), or by mitotic division at the boundary of the zonae glomerulosa and fasciculata (Hoerr,[26] Gruenwald and Konikov,[27] Gruenwald[8]). The dye injection experiments of Salmon and Zwemer[28] have been vigorously attacked by Calma and Foster,[29] McPhail,[30] and Baxter.[31] The objections of the latter authors are undoubtedly correct. The necessity of the capsule for regrowth of the cortex in transplantation has been established by Turner,[32] Ingle and Higgins,[33] and by Baker and Baillif.[34] Williams[35,36] concluded that regeneration in adrenal cortical grafts begins in the glomerular region, and possibly in the capsule. Blackman[37] thought that the normal post-natal reticular zone originates at an early age from persistent cells of the fetal reticular zone which have failed to undergo involution.

The concept that cell replacement in the adrenal cortex takes place by proliferation of the peripheral zone with the zona reticularis representing a senescent zone had been enunciated by Canalis[38] in 1887, and by Mulon,[39,40] and Celestino Da Costa.[41] Later work described more accurately the mode of incorporation of new cells into the cortex, either by end-to-end junction (Zwemer, Wotton, and Norkus[24]), or by apposition from the capsule (Gruenwald and Konikov[27]). Both methods were observed in the material studied.

The observations of this study are consistent with the double mode of growth of cortical cells once they have penetrated into the peri-adrenal fat tissue, namely, by apposition from the capsule and/or by transformation of glomerulosa cells into fasciculata cells. The evidence is insufficient to indicate which mode is more important. Our material furnishes no evidence for the assumption that undifferentiated connective tissue elements in the peri-adrenal fat may, by differentiation *in situ*, become incorporated into the growing mass. The complete absence of nonencapsulated masses in the peri-adrenal fat of the newborn infant is worthy of note, but at present cannot be explained.

Cells of the zona reticularis, as mentioned, were identified in 12 (16.7 per cent) cases. This finding has no relation to the number of sections examined per case. In 5 of the 12 cases there were 5 or less sections. In only 2 cases was the number of cells commensurate with the size of the mass. In the rest they were isolated and few, identified

chiefly by their pigment. In view of these findings, the supposed life cycle of capsule→glomerulosa→fasciculata→reticularis does not necessarily represent the sequence of events in the extracapsular masses. It is possible that these cells differ functionally from those within the capsule; nothing is known about their life span.

Conclusions

In 72 of 257 adult adrenal glands, nonencapsulated adrenal cortical tissue was found free in the peri-adrenal fat. Such collections were not found in 36 newborn infants.

The positive cases increase moderately with advancing age.

There was no correlation with any other clinical or anatomic data.

Cells of the zona reticularis were found in only 12 of the 72 positive cases, suggesting that the usually accepted life cycle of cortical cells may not apply to the extracapsular masses and that the latter may differ functionally from the normal cortex.

I wish to thank Dr. Alfred Plaut for suggesting the subject, and for his painstaking guidance during the preparation of this paper.

REFERENCES

1. Henle, F. G. J. Allgemeine Anatomie. Lehre von den Mischungs- und Formbestandtheilen des menschlichen Körpers. L. Voss, Leipzig, 1841, ed. 1, 1048 pp.

2. Huschke, E. Traité de splanchnologie et des organes des sen. In: Encyclopédie anatomique. (Translated by A. Jourdan.) J. B. Baillière, Paris, 1845, **5**, 870 pp.

3. Kölliker, A. v. Mikroskopische Anatomie, oder Gewebelehre des Menschen. W. Engelmann, Leipzig, 1852, **2**, 784 pp.

4. Cornil, V., and Ranvier, L. Manuel d'histologie pathologique. J. B. Baillière, Paris, 1869, 1265 pp.

5. Testut, L. Traité d'anatomie humaine. O. Doin, Paris, 1895, ed. 3.

6. Soulié, A. H. Recherches sur le développement des capsules surrénales chez les vertébrés supérieurs. *J. de l'anat. et physiol.*, 1903, **39**, 197–293; 390–425; 492–533; 634–662.

7. Broster, L. R., and Vines, H. W. C. The Adrenal Cortex. H. K. Lewis & Co., Ltd., London, 1933.

8. Gruenwald, P. Embryonic and postnatal development of the adrenal cortex, particularly the zona glomerulosa and accessory nodules. *Anat. Rec.*, 1946, **95**, 391–421.

9. Kovács, W. Zur Nebennierenpathologie. *Beitr. z. path. Anat. u. z. allg. Path.*, 1928, **79**, 213–267.

10. Huebschmann. Beiträge zur pathologischen Anatomie der Nebennieren. (Atrophie und Hypertrophie.) *Beitr. z. path. Anat. u. z. allg. Path.*, 1921, **69**, 352–365.

11. Kaiserling, C. Missbildung und verborgene Tuberkulose der Nebennieren eines Erwachsenen. *Berl. klin. Wchnschr.*, 1917, **54**, 79–82.

12. Sudds, M. V. N. The cell contents of the cortex of the suprarenal gland. *Endocrinology*, 1940, **26**, 895–899.

13. Goldzieher, J. W. The adrenal glands in pemphigus vulgaris. *Arch. Dermat. & Syph.*, 1945, **52**, 369–375.

14. Plaut, A. Discussion of J. W. Goldzieher's paper. *Proc. New York Path. Soc.*, 1945, pp. 151–152.

15. Goormaghtigh, N. Le cortex surrénal humain dans les plaies de l'abdomen et aux périodes intéressantes de la vie sexuelle. Thèse, Université de Gand, Liège, 1922, 90 pp.

16. Rogoff, J. M. The Suprarenal Bodies. In: Cowdry, E. V. (ed.) Special Cytology. Paul B. Hoeber, Inc., 1932, ed. 2, **2**, 871–931.

17. Zwemer, R. L. A study of adrenal cortex morphology. *Am. J. Path.*, 1936, **12**, 107–114.

18. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia & London, 1942, ed. 4, 695 pp.

19. Bailey, F. R. Textbook of Histology. (Rev. by Smith, P. E., and Copenhaver, W. M.) Williams & Wilkins Co., Baltimore, 1944, ed. 11, 786 pp.

20. Cowdry, E. V. A Textbook of Histology. Lea & Febiger, Philadelphia, 1944, ed. 3, 426 pp.

21. Bennett, H. S. The life history and secretion of the cells of the adrenal cortex of the cat. *Am. J. Anat.*, 1940, **67**, 151–227.

22. Yeakel, E. H. Medullary hyperplasia of the adrenal glands in aged Wistar albino and gray Norway rats. *Arch. Path.*, 1947, **44**, 71–77.

23. Wotton, R. M., and Zwemer, R. L. A study of the cytogenesis of cortico-adrenal cells in the cat. *Anat. Rec.*, 1943, **86**, 409–416.

24. Zwemer, R. L., Wotton, R. M., and Norkus, M. G. A study of corticoadrenal cells. *Anat. Rec.*, 1938, **72**, 249–263.

25. Elias, H. Cortical cell replacement in the adrenal gland of domesticated ungulata. *Anat. Rec.*, 1947, **99**, 609.

26. Hoerr, N. The cells of the suprarenal cortex in the guinea-pig. Their reaction to injury and their replacement. *Am. J. Anat.*, 1931, **48**, 139–197.

27. Gruenwald, P., and Konikov, W. M. Cell replacement and its relation to the zona glomerulosa in the adrenal cortex of mammals. *Anat. Rec.*, 1944, **89**, 1–21.

28. Salmon, T. N., and Zwemer, R. L. A study of the life history of corticoadrenal gland cells of the rat by means of trypan blue injections. *Anat. Rec.*, 1941, **80**, 421–429.

29. Calma, I., and Foster, C. L. Trypan blue and cell migration in the adrenal cortex of rats. *Nature, London*, 1943, **152**, 536.

30. McPhail, M. K. Trypan blue and growth of the adrenal cortex in mice. *Nature, London*, 1944, **153**, 460–461.

31. Baxter, J. S. The growth cycle of the cells of the adrenal cortex in the adult rat. *J. Anat.*, 1946, **80**, 139–146.

32. Turner, C. D. Homotransplantation of suprarenal glands from prepuberal rats into the eyes of adult hosts. *Anat. Rec.*, 1939, **73**, 145–162.

33. Ingle, D. J., and Higgins, G. M. Autotransplantation and regeneration of the adrenal gland. *Endocrinology*, 1938, **22**, 458–464.

34. Baker, D. D., and Baillif, R. N. Rôle of capsule in suprarenal regeneration studied with aid of colchicine. *Proc. Soc. Exper. Biol. & Med.*, 1939, **40**, 117–121.

35. Williams, R. G. The characteristics and behavior of living cells in autogenous grafts of adrenal cortex in rabbits. *Am. J. Anat.*, 1945, **77**, 53–79.

36. Williams, R. G. Studies of adrenal cortex: regeneration of the transplanted gland and the vital quality of autogenous grafts. *Am. J. Anat.*, 1947, **81**, 199–231.

37. Blackman, S. S., Jr. Concerning the function and origin of the reticular zone of the adrenal cortex. *Bull. Johns Hopkins Hosp.*, 1946, **78**, 180–216.

38. Canalis, P. Contribution à l'étude du développement et de la pathologie des capsules surrénales. *Internat. Monatschr. f. Anat. u. Physiol., Leipz.*, 1887, **4**, 312–334.

39. Mulon, P. Divisions nucléaires et rôle germinatif de la couche glomérulaire des capsules surrénales du cobaye. *Compt. rend. Soc. de biol.*, 1903, **55**, 592–595.

40. Mulon, P. Évolution de la corticale surrénale du cobaye avec l'age de l'animal. *Compt. rend. Soc. de biol.*, 1905, **59**, 337–339.

41. Celestino Da Costa, A. Recherches sur l'histophysiologie des glandes surrénales. *Arch. de biol., Paris*, 1913, **28**, 111–196.

HISTOPATHOLOGY OF OVARIAN PSEUDOMUCINOUS CYSTADENOMA*

JAMES W. REAGAN, M.D.

(From the Institute of Pathology, Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio)

A varied terminology, now only of historic value, has been applied for more than a century to the pseudomucinous cystadenoma. The term pseudomucin was originally used to describe the content of the locules, which was thought to differ from mucin. Chemically, both mucin and pseudomucin are composed largely of glycoproteins, and differ only in certain physical characters. The exact nature of the secretion is not of diagnostic importance because the tumor is identified by its gross and microscopic features. The literature has been covered by Selye[1] and need not be reviewed further. Pertinent references are made throughout the paper.

This investigation is a detailed histopathologic study of 120 pseudomucinous cystadenomas, with particular attention to the epithelial component.

MATERIALS AND METHODS

The material consists of surgical specimens submitted to the Institute of Pathology of the University Hospitals. Only typical tumors with characteristic epithelium are included in the study. Formalin-fixed material was utilized for the most part, but in a few instances fresh specimens were preserved in special fixatives. Each tumor was examined with hematoxylin and eosin preparations. Numerous special stains were employed as will be indicated during the course of this report.

PATHOLOGIC ANATOMY

There was a wide variation in the size of the tumors in this series. The smallest was made up of only a few microscopic locules, while the largest specimen weighed over 22 kg. The cystadenomas usually ranged in weight from 800 to 1500 gm. In only two instances were they bilateral. Some were pedunculated.

Grossly, the cystadenoma consisted of a variable number of locules surrounded by connective tissue. The tumor might be solid with abundant intervening stroma separating small and more or less uniform locules, as in the parvilocular form discussed by Schiller.[2] In the more common multilocular form the locules were larger, more variable in size, and separated by thin connective tissue septa. Mixed forms also occurred. The confluence of smaller locules might eventually lead to the production

* Received for publication, August 13, 1948.

of a predominantly unilocular cystoma, although careful examination disclosed small locules within the capsule. Any of the forms might be papilliferous. This series did not include examples of cystadenoma of the so-called racemous type, in which the tumor resembles "grapes" supported on connective tissue stalks.

The tumor locules contained viscid, transparent or translucent fluid which in some sites was inspissated and firm. With hemorrhage from ruptured vessels, the fluid might be dark red, brown, or black. In the presence of necrosis the contents were yellow and grumous. With necrosis in combination with cholesterol the color might be green.

Capsule

A well defined connective tissue capsule surrounded the cystadenoma except at the ovarian hilum where the capsule was poorly defined or absent. Connective tissue laminae of varying thickness and density made up the capsule, which might also contain spindle-shaped cells resembling smooth muscle but lacking myofibrils in their cytoplasm. Kuge[8] believed smooth muscle exists in the capsule. In agreement with Doran,[4] dense bundles of elastic fibers were found to occur in the capsule, and in the interlocular septa. Their presence probably was the result of displacement from the medulla of the ovary. While there were wide variations in the vascularity of the capsule, larger vessels usually lay in the mid-portion of the connective tissue zone, and numerous capillaries might exist beneath the locular epithelium. Calcification, ossification, edema, inflammation, degeneration, or necrosis altered the pattern of the connective tissue. In the capsules of smaller tumors there might be compressed ovarian tissue.

The innermost connective tissue lamina was situated immediately outside the basement membrane of the locule, and might be thin, hyalinized, and poorly defined. Infrequently, zones or groups of polyhedral epithelioid cells, with clear cytoplasm containing small droplets of fat as demonstrated with sudan IV, surrounded the basement membrane. Between the cell groups were numerous capillaries. In some sites a poorly defined zone of compressed spindle-shaped cells surrounded the locule. Frequently, fat in the form of small droplets of variable size was detected in these cells. Adjacent stromal cells sometimes contained fat droplets of a much smaller size.

Basement Membrane

Between the epithelium and the underlying stroma there was an argyrophilic membrane of variable width and definition. In the younger locules the continuity of the membrane was maintained, but in the larger

loculesof mature tumors it usually was broken at many sites, probably because of the rapid enlargement of the locules. The basement membrane presumably was produced by a condensation of the underlying reticulum, and was poorly defined or absent and without surrounding reticular fibers. Fox[5] was unable to demonstrate a membrana limitans, but Malassez and de Sinéty[6] spoke of the presence of an incomplete subepithelial endothelium. The presence or absence of such a structure might well depend on the authors' definition of a basement membrane.

Locules

The locules of the smallest tumors usually were situated beneath the free border of the ovary in the innermost cortical zone away from the hilum of the ovary, but might occur in the medulla adjacent to the cortex. Such primitive locules of uniform or variable size were lined with simple cuboidal or columnar epithelium. Pseudopapillae were common in locules lined by columnar cells. Slightly larger primitive locules of a more irregular outline were lined by simple flat epithelium with gradual transition to tall-columnar cells. On serial section most of these locules were isolated.

The presence of simple flat epithelium in such locules is of some importance. In view of columnar cells elsewhere in the locules, and the irregular outline of the locules, it is unlikely that the existing intralocular pressure is sufficient to cause pressure atrophy. The irregular outline of the locules and the presence of flat epithelium may be interpreted as evidence that such locules were previously under much greater tension.

The cytoplasm of the cuboidal and columnar epithelium stained with mucicarmine in each instance, while that of the flat epithelium stained poorly, if at all. Rarely, islands of small polyhedral cells might be identified in the lining epithelium or in the locular lumina. In no position did the primitive locules approximate the tubules of the rete ovarii, epoophoron, or paroophoron.

In large tumors the locules were more variable in size and distribution and there was no correlation between the size of the locule and the height of the lining epithelium. The cells might be cuboidal, columnar, or flat, with transition to columnar cells. Transition from flat to columnar epithelium was less often observed in larger tumors. The intralocular tension in large locules might result in pressure atrophy of the epithelium. In locules lined with goblet cells, the degree of epithelial compression was evident. In such epithelium the long axis of the goblet was parallel with the basement membrane, rather than vertical. While in the flat epithelium of the primitive locule there were few or no secretory granules

staining with mucicarmine, that of the larger locules contained numerous granules reacting with this stain. In proliferation the epithelium might be tall and prismatic, and an extreme degree of proliferation was present in the epithelium of a cystadenoma removed during pregnancy. Spurs or remnants of septa occurred in the larger locules of older tumors. Multiplication of locules occurred by inclusions of locular epithelium, and in this way satellite locules were formed. In one instance there was a corpus albicans in the wall of a locule. Over the festooned hyalinized mass the epithelium was intimately applied. The cells were flat with transition to tall-columnar epithelium, and numerous secretory granules were demonstrated with mucicarmine. It was impossible to ascertain whether such a locule arose from a corpus albicans cyst, although such cysts have been reported lined by tall-columnar epithelium. The epithelium might be altered by necrosis, degeneration, or inflammation. Within the locules there might be lymphocytes, polymorphonuclear leukocytes, desquamated epithelium, macrophages or cholesterol crystals.

Columnar Epithelium

The epithelium most characteristic of the pseudomucinous cystadenoma was of simple columnar type. Pseudo-stratification usually was the result of a tangential plane of section, but foci of true stratification exist. Even though Novak[7] held that the cells are always simple columnar, Shaw[8] found stratification at some place in 24 per cent of 91 specimens.

The columnar cells measured from 10 to 54 μ in height and 3 to 8 μ in width. The size and shape of the individual cell depended on the pressure exerted upon it, the intracellular activity, and the position of the cell in the epithelial layer. The pressure of the intralocular contents of mature tumors might depress the epithelium. In addition, the pressure of adjacent epithelial cells might alter the shape of the cell. While most, if not all, of the cells could be transformed into goblet or chalice cells, their lateral borders frequently remained more or less parallel owing to the pressure of contiguous cells. As will be discussed later, the morphologically typical goblet cells were present in sites where pressure was not great.

The secreting epithelium underwent cyclic alterations with characteristic changes in the cellular configuration. Barzilai[9] recognized separate phases of intracellular activity, and defined secretory, excretory, and resting phases. Her description of the secretory and excretory phases is in keeping with the results of this study, but the description of the resting phase differs somewhat from my findings in this stage. With increasing intracellular secretion the cell might become wider or slightly

pyramidal. On discharge of the secretion the lateral cell borders might become compressed because of the pressure of adjacent cells. There was no evidence that the cells regressed in height after completion of a cycle. Adjacent cells might vary in their phases of activity, and the epithelium of the locule did not necessarily exhibit a uniform cyclic change as is true of the endometrium. Thus there was no apparent correlation between the endometrial cycle and the phase of the cellular activity in this tumor. Comparison was possible in only a few instances since curettage was not usually performed at the time of operation.

The configuration of the cells was altered by fatty degeneration, cloudy swelling, and in proliferation. However, such pleomorphism should not be interpreted as evidence of carcinoma.

Terminal Bars

Terminal bars were demonstrated in Heidenhain's iron-hematoxylin preparations. These were in or on the free border and closed the intercellular clefts on the free surface. When section was horizontal through the free border, the bars appeared as solid bands encircling the cells, but when the section was vertical they were in the form of small nodes or bars at the free border. Maximow and Bloom[10] stated that they are composed of a cementing substance. This may explain the strong cohesiveness of the cells and the fact that they are characteristically more adherent to one another than to the underlying tissues.

Striate Border

A striate border was demonstrated only infrequently on the free margin of the epithelium. It was apparent with hematoxylin and eosin and Heidenhain's iron-hematoxylin staining. The striate border occurred on actively secreting epithelium and consisted of a fine, regular, vertical striation measuring up to 2.5 μ in height. Beneath the striate border was a transverse dark line which in some sites could be resolved into small basal granules. Because of the limited number of fresh specimens and the infrequent occurrence of the structure, examination by fresh tissue was not done. Characteristically, however, the striate border does not possess motility. Such a structure is present on intestinal epithelium and has been demonstrated on the epithelium of the placental villus. A comparable structure, *i.e.*, the brush border, is characteristic of the epithelium of the renal tubules. The exact nature of the structure is unknown, although it has been suggested that the striations are the structural expression of water currents entering or leaving the cell. Lucas[11] believed that it is not genetically related to the ciliary apparatus.

Ciliated Epithelium

Careful examination of the fixed preparations in this series revealed no epithelial cilia. This is in accordance with the observations of Shaw[8] and Geist,[12] but Miller[13] and Novak[7] claimed to have demonstrated ciliated epithelium. As previously noted, fresh specimens were not examined for the presence of cilia.

Cell Membrane

The columnar cells possessed a well defined cell membrane. The membrane overlying the free margin of the cell reflected the intracellular activity. Thus, when the amount of secretion was small, the border was flat, but with larger amounts of secretion and increased intracellular tension, it bulged. With discharge of the cell contents, the membrane stained poorly and ruptured. In other cells a small stoma appeared in the cell membrane, which extended through the striate border when that was present. In those cells with a well defined stoma the excretion appeared to occur slowly and a plug of secretion might persist in the stoma. In the tall prismatic epithelium of proliferation, the cell membrane appeared to be wrinkled, but this may be an artifact.

Nuclei

The nuclei varied in size, shape, and intracellular position. In low-columnar epithelium the nucleus might be spherical, averaging 5.5 μ in diameter, while in taller columnar epithelium the nuclei were ovoid and averaged 8 μ in greatest diameter. Elongated compressed nuclei measured up to 10 μ in greatest dimension. In the resting cell the nucleus usually was ovoid and was in the basal aspect of the middle third of the cell. At this stage the nuclear chromatin network was only slightly basophilic and the nucleolus was small, but with increasing secretion the chromatin network became more deeply basophilic and one or, rarely, two prominent nucleoli might be observed. As secretion accumulated in the cell there developed a depression in the superior nuclear pole, and eventually the nucleus might be displaced basally in the cell to be converted into a cup-shaped mass. Other nuclei became flattened and basal in location. Some cells with a poorly defined streamer extending from the inferior nuclear pole to the cell base perhaps were less subject to nuclear displacement. With discharge of the cell secretion, the nuclei became ovoid and returned to the resting position.

The nuclear infolding or grooving in the longitudinal plane was observed especially in tall proliferating cells. Danforth[14] believed that it is more common in lower epithelium. According to Arey,[15] this nuclear

folding was first observed by Varangot, but Danforth was first to identify it in the pseudomucinous cystadenoma. This nuclear change is not specific and occurs in follicular epithelium, endocervical epithelium, and may occur in the cells of Sertoli as well as in other sites.

When studied with Heidenhain's iron-hematoxylin stain the nucleus had a well defined nuclear membrane. A chromatin network was present, sometimes containing small chromatin masses. Although mitotic figures were not often observed, they became numerous when proliferation was active.

Cytoplasm

The cytoplasm usually was diffusely and finely granular, and the granules might be either acidophilic or basophilic. I agree with other investigators that the granules are primarily acidophilic and subsequently become basophilic. As the granules enlarged and were distributed more widely in the cell, they appeared to lie against a clear cytoplasmic background to give the so-called ground glass appearance. The mucicarmine stain was used to study the secretory granules of the epithelium. It should be remembered that while this stain is of some aid in identifying tumors of this type, the cells of the serous cystadenoma may also contain secretory granules reacting with mucicarmine. The granules varied somewhat in their distribution throughout the cycle. In the early stage they were small and supranuclear in location, later filling the theca of the goblet cells and producing a more or less confluent mass of secretion. With increasing secretion the cell cytoplasm became compressed and a poorly defined basophilic zone was apparent about the basal nuclei.

Many of the cells had a demonstrable chromophil substance. With toluidine blue such cells had vertical basal striations, usually situated beneath the nucleus but in some cells extending up about the nucleus. While the origin and function of the chromophil substance is unknown, it is similar to the Nissl substance of nerve cells.

With metachromatic dyes such as cresyl violet and crystal violet, some of the secretory granules exhibited a true metachromatic reaction. These granules were slightly larger and better defined than other granules, and might be observed both within the epithelium and in the locular lumina. In view of the possible entodermal origin of the cystadenoma, some may consider these cells as being similar to those of Paneth. Hertzog,[16] in studying the cells of Paneth, employed de Galantha's amyloid stain,[17] and found the granules to stain similarly to amyloid. While Congo red was employed by Hertzog, its use here was unsatisfactory because of the poor differentiation of the stain. Morphologically, the

granules staining with metachromatic dyes were not unlike those of many types of mucus-producing epithelium. The presence of such secretory granules in goblet cells will be described later.

In addition, when stained with sudan IV, the cytoplasm of the columnar cells contained many minute, finely dispersed fat droplets. With sudan black B there were comparable black granules. This finding was common and is probably not a manifestation of degeneration, because in degeneration the demonstrable fat occurs in larger droplets distributed both throughout the cell and beneath the nucleus.

Only a few specimens were examined for glycogen with Best's carmine technic. A few of the cells contained well defined droplets of glycogen distributed irregularly throughout the cytoplasm.

Mitochondria

Mitochondria were demonstrated with Kull's[18] modification of the Altmann technic. The mitochondria of the columnar epithelium usually were filamentous. The filaments consisted of long, thin, rod-like structures arranged more or less parallel to the long axis of the cell. They might be interwoven and wavy, and extended from the cell base to a site beneath the free border. The filaments adjacent to the nucleus might follow the curvature of the nuclear poles or even encircle the nucleus.

Granular forms of mitochondria also occurred. They are not to be confused with filaments sectioned in a tangential plane. Granular forms usually were below the nucleus but might be above it also

In the normal cycle, mitochondrial changes were slight or absent, but in degeneration they were noteworthy. In the degenerate cell the mitochondria became fragmented and appeared as short rods and granules without orientation in the cell. In other degenerate cells mitochondria were absent, having undergone chondriolysis. These observations are in keeping with those of Cowdry.[10]

The only conclusions drawn from examination of the mitochondria are as to their type and changes in degeneration. The multiplicity of technical errors in studying such structures makes evaluation difficult.

Goblet Cells

Typical goblet cells occurred in sites where compression was not great. They were numerous in some of the locules and were common over the papillae. Within the theca of the cells there were secretory granules staining with both mucicarmine and the metachromatic dyes. These granules were large, and with Heidenhain's iron-hematoxylin stain were well defined and surrounded by a small, clear halo. A small stoma might

appear in the theca and through this opening the secretion was discharged. Beneath the nucleus there might be a thin projection anchoring the cell to the underlying basement membrane. The goblet cells were not in phase with the adjacent columnar cells and appeared to have their own independent cycle. After discharge of the cell content, the theca collapsed and the cell might become compressed between adjacent columnar cells.

With rapid proliferation of the columnar epithelial layer, these goblet cells might be forced upward and the anchoring streamer broken. Such cells lay high in the cell layer and in serial sections presented no demonstrable connection with the basement membrane, but they still might undergo a cycle in this site. The nuclei of such cells were spherical, deeply basophilic, and lay beneath the free border of the columnar epithelium. In secretion, a small supra-nuclear vacuole was formed. This enlarged and eventually the nucleus was converted into a crescentic mass with overlying theca containing secretion. Rarely there was discharge of the entire cell, including the nucleus and cytoplasm, and thus a discharge of partial holocrine type was present in addition to merocrine secretion. Varying degrees of alteration in polarity might be present, with secretion even occurring beneath the nucleus in some cells. The secretory granules were similar to those of the goblet cells lower in the epithelium.

Between the nuclei of the columnar cells and the basement membrane there frequently were irregular nuclear masses. These averaged 6 μ in diameter, were deeply basophilic, and frequently were surrounded by a clear zone of variable width which contained no granules in mucicarmine preparations. Such cells represented leukocytes or perhaps degenerate tumor cells.

Less often in this basal region there were secreting cells with loss of polarity. These were goblet cells which had been displaced downward in the epithelium. Even with rupture of the basement membrane these cells were not detected invading the stroma.

Argentaffin Cells

Argentaffin cells were first demonstrated in this tumor by Masson.[20] Similar cells in the intestinal tract are spoken of as enterochromaffin cells, basal granular cells, or argentaffin cells. We use the term argentaffin as descriptive of the granules, without implying that the cells are of intestinal origin.

As demonstrated by Masson's technic, the cells were perhaps more common in the parvilocular tumors, but even in that type of cystadenoma

they were not numerous. The cells usually were widely scattered and did not occur side by side. The cell itself might be flattened or flask-shaped, located between adjacent columnar cells or compressed against the basement membrane. The cell nucleus was spherical, averaging 7 μ in diameter, and might be situated within or above the granular mass. The surrounding argentaffin granules were small and so closely packed in some cells that the individual granule could not be outlined. The granules varied somewhat in their staining reaction with the silver technic, and both black and brown granules occurred, as noted by Masson[21] in paraffin sections.

The chemical characteristics of the enterochromaffin cells of the intestinal tract have been investigated by Gomori,[22] who believed the granules contain a derivative of resorcinol. According to Macklin and Macklin,[23] similar cells have been identified in the esophagus, stomach, intestine, adrenal, and pancreas.

The origin of the argentaffin cells in this tumor is unexplained. Witschi[24] recently has confirmed the observations of earlier investigators in regard to a migration of the germ cells to the anlage of the gonad. In this study Witschi stated that there is no evidence that other cells accompany the migrating germ cells. The presence of such argentaffin cells in this tumor is not a substantial basis for postulating enteric origin. Further study of these cells is now in progress.

Histogenesis

The literature dealing with the genesis of this tumor is extensive, and since the earlier works are speculative rather than investigative, detailed reference is unjustified. Pflüger's tubules have been implicated by some, but Felix[25] concluded that such tubules do not exist in man. Forbes,[26] in a study of the medullary cords of the human ovary, found only one "medullary tubule" in 55 ovaries examined. He stated further that the medullary cords usually disappear in fetuses of 280 mm. crown-rump length. The tubules of the epoophoron and rete ovarii also have received attention. Goodall[27] suggested the possibility of origin from the germinal epithelium, and also emphasized the association of the tumor with teratomas of the ovary. Bland-Sutton[28] referred to the cystadenomas as embryomas in which gastro-intestinal epithelium predominates. Schiller[29] and most of the contemporary group classify the tumor as a monophyletic teratoma arising from displacement of cells during embryonal life. Novak and Jones[30] suggested that some of the cystadenomas may arise from Brenner tumors. Origin from follicular epithelium is postu-

lated by many writers, but Gardner[31] probably was the first to demonstrate it.

The three smallest cystadenomas in this series, consisting of only a few microscopic locules, were found in ovaries of the polycystic type. The locules were situated in the innermost cortical zone (zona vasculosa) corresponding to the site of earliest follicular maturation, and most of these on serial section were isolated, suggesting but not proving a multicentric origin of the tumor.

One type of primitive locule, common to each of the small tumors and identical with that described by Shaw,[32] is lined by flat epithelium with gradual transition to columnar epithelium. The simple flat epithelium of such locules is not unlike that lining the microcystic follicle in atresia, and contains few or no secretory granules when stained with mucicarmine. Rarely, islands of small polyhedral cells morphologically resembling granulosa occur in the locular epithelium or in the locular lumina. A well defined zone of granulosa as demonstrated by Gardner[31] was not observed in this study. Neither a degenerate ovum nor fragments of zona pellucida were present in any of the locules.

Outside the basement membrane of some locules there was a zone of polyhedral cells with ovoid or spherical nuclei and clear cytoplasm containing numerous droplets of fat. Between the cells of this zone there were numerous capillaries extending to the basement membrane. This zone was morphologically similar to the theca lutein layer of the follicle in regression. Outside other locules there were connective tissue cells containing droplets of fat of variable size, also suggesting thecal origin. Other locules without such features may represent satellite locules. On the basis of these observations in the primitive locules, origin of the tumor in the atretic follicle is strongly suggested.

The current view is that degeneration of the ovum precedes follicular atresia, although Novak[33] once suggested that it depends on discharge of the ovum. Atresia may occur at any stage of follicular development, and Bartelmez,[34] in describing the series of events, stated that the granulosa nearest the atrium of the follicle undergoes degeneration first, while that near the basement membrane degenerates later. Such involution results in the formation of microcystic follicles lined by flat or cuboidal epithelium with or without recognizable granulosa. Such follicles are frequent in the ovary.

In studying ovaries of various age groups, I have found that the liquor folliculi and the zona pellucida of the atretic follicle may stain with mucicarmine. Similarly, with this stain the granulosa cells may show

UNIVERSITY of PENNSYLVANIA LIBRARIES

cytoplasmic secretory granules. This similarity in staining reaction is not unusual since both the zona pellucida and the liquor folliculi are products of the granulosa cells. The germinal epithelium of infant ovaries contains no secretory granules with mucicarmine, but in the adult this layer may stain with mucicarmine.

Serial sections of the smallest tumors revealed no continuity of the germinal epithelium and the locular epithelium. According to Wilson,[35] the human rete ovarii extends only a short distance into the gonad arising from the most deeply lying cells of the early sex gland. Thus there is no reason to suspect origin from the rete tubules, and in no site do the primitive locules approximate these structures. In only one specimen was there a coexisting Brenner tumor, and there is no evidence to suggest origin from this tumor. The association of the cystadenoma with teratomas is of such infrequency as to be inconsequential.

The exact cause of the prosoplastic metaplasia of the epithelium in the production of this tumor is unknown. That the stimulus is hormonal in nature is suggested by the rapid rise in the incidence of the tumor after puberty and its occurrence during the active sexual periods of life.

The epithelium of the cystadenoma is compared to that of the gallbladder, stomach, intestine, and endocervix by other authors. It is similar to other mucus-secreting epithelium in the body, and more nearly resembles intestinal epithelium.

Summary and Conclusions

The pseudomucinous cystadenoma is a multilocular or unilocular neoplasm lined by epithelium which has the properties of secretion and proliferation. The locules of variable size and number usually are lined by simple columnar epithelium which may have a striate border. In addition, goblet cells, argentaffin cells, and simple flat and cuboidal epithelium may be present.

In the presence of merocrine secretion the epithelium undergoes a cyclic alteration. A secretion of partial holocrine type also occurs in some cells. The secretory granules of the epithelium stain with mucicarmine, and in some instances with metachromatic dyes such as cresyl violet and crystal violet. Within the cellular cytoplasm there may be demonstrable fat, glycogen, and a chromophil substance. The mitochondria are of the filamentous type and may be granular. Mitochondria become fragmented in cellular degeneration.

Evidence is presented to suggest the follicular origin of the cystadenoma. In the development of the tumor either secretion or proliferation may dominate, or the two processes may be in balance.

Enlargement of tumor locules results in a reduction of number and eventually in the formation of a unilocular cystoma.

I wish to express my appreciation to Miss Ethel Lieb for expert technical assistance in this study.

REFERENCES

1. Selye, H. Encyclopedia of Endocrinology. Richardson, Bond & Wright, Montreal, 1946, 7, Sect. 4, 98–142.
2. Schiller, W. Parvilocular tumors of the ovary. *Arch. Path.*, 1943, 35, 391–413.
3. Kuge, K. Ueber die Knockenkörper in den pseudomucinösen Ovarialkystomen. *Mitt. ü. allg. Path. u. path. Anat.*, 1923, 2, 41–63.
4. Doran, A. Clinical and Pathological Observations on Tumours of the Ovary, Fallopian Tube, and Broad Ligament. Smith, Elder, & Co., London, 1884, pp. 14–39.
5. Fox, W. On the origin, structure, and mode of development of the cystic tumours of the ovary. *Med. Chir. Trans.*, 1864, 47, 227–287.
6. Malassez and de Sinéty. Sur la structure, l'origine et le développement des kystes de l'ovaire. *Arch. de physiol. norm. et path.*, 1879, 6, s. 2, 624–654.
7. Novak, E. Gynecological and Obstetrical Pathology. W. B. Saunders Co., Philadelphia, 1947, ed. 2, pp. 326–343.
8. Shaw, W. The pathology of ovarian tumours. *J. Obst. & Gynaec. Brit. Emp.*, 1932, 39, 234–266.
9. Barzilai, G. Atlas of Ovarian Tumors. Grune & Stratton, New York, 1943, pp. 203–223.
10. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia & London, 1942, ed. 4, pp. 35–36.
11. Lucas, A. M. Ciliated Epithelium. In: Cowdry, E. V. Special Cytology. Paul B. Hoeber, Inc., New York, 1932, 1, 409–474.
12. Geist, S. H. Ovarian Tumors. Paul B. Hoeber, Inc., New York, 1942, pp. 154–171.
13. Miller, J. Die Krankheiten des Eierstockes. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1937, 7, Pt. 3, 502–511.
14. Danforth, D. N. The cytologic relationship of the Walthard cell rest to the Brenner tumor of the ovary and to the pseudomucinous cystadenoma. *Am. J. Obst. & Gynec.*, 1942, 43, 984–996.
15. Arey, L. B. The nature and significance of the grooved nuclei of Brenner tumors and Walthard cell islands. *Am. J. Obst. & Gynec.*, 1943, 45, 614–624.
16. Hertzog, A. J. The Paneth cell. *Am. J. Path.*, 1937, 13, 351–360.
17. de Galantha, E. A new stain for connective tissue, mucin, and allied substances. *Am. J. Clin. Path.*, 1936, 6, 196–197.
18. Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia & London, 1938, p. 111.
19. Cowdry, E. V. The reactions of mitochondria to cellular injury. *Arch. Path.*, 1926, 1, 235–255.
20. Masson, P. Sur la présence de cellules argentaffines dans les kystes pseudomucineaux de l'ovaire. *Union méd. du Canada*, 1938, 67, 2–5.
21. Masson, P. Carcinoids (argentaffin-cell tumors) and nerve hyperplasia of the appendicular mucosa. *Am. J. Path.*, 1928, 4, 181–211.
22. Gomori, G. Chemical character of the enterochromaffin cells. *Arch. Path.*, 1948, 45, 48–55.
23. Macklin, C. C., and Macklin, M. T. The Intestinal Epithelium. In: Cowdry, E. V. Special Cytology. Paul B. Hoeber, Inc., New York, 1932, 1, 268–302.

24. Witschi, E. Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. *Contrib. Embryol.*, 1948, **32**, No. 209.
25. Felix, W. The Development of the Urinogenital Organs. In: Keibel, F., and Mall, F. Manual of Human Embryology. J. B. Lippincott Co., Philadelphia & London, 1912, **2**, 904.
26. Forbes, T. R. On the fate of the medullary cords of the human ovary. *Contrib. Embryol.*, 1942, **30**, No. 188.
27. Goodall, J. R. The origin of epithelial tumours of the ovary. *Proc. Roy. Soc. Med.* (Sect. Obst. & Gynaec.), 1919–20, **13**, 63–95.
28. Bland-Sutton, J. Bionomics of animal reproduction in relation to tumours and cancers. *Lancet*, 1927, **2**, 741–743.
29. Schiller, W. Concepts of a new classification of ovarian tumors. *Surg., Gynec. & Obst.*, 1940, **70**, 773–782.
30. Novak, E., and Jones, H. W. Brenner tumors of the ovary. *Am. J. Obst. & Gynec.*, 1939, **38**, 872–888.
31. Gardner, W. S. On the origin of ovarian epithelium. *Am. J. Obst. & Gynec.*, 1932, **23**, 54–61.
32. Shaw, W. The pathology of ovarian tumours. *J. Obst. & Gynaec. Brit. Emp.*, 1933, **40**, 257–272.
33. Novak, E. The corpus luteum; its life cycle and its rôle in menstrual disorders. *J. A. M. A.*, 1916, **67**, 1285–1291.
34. Bartelmez, G. W. Female Genital System. In: Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia & London, 1942, ed. 4, pp. 554–592.
35. Wilson, K. M. Origin and development of the rete ovarii and the rete testis in the human embryo. *Contrib. Embryol.*, 1926, **17**, No. 86.

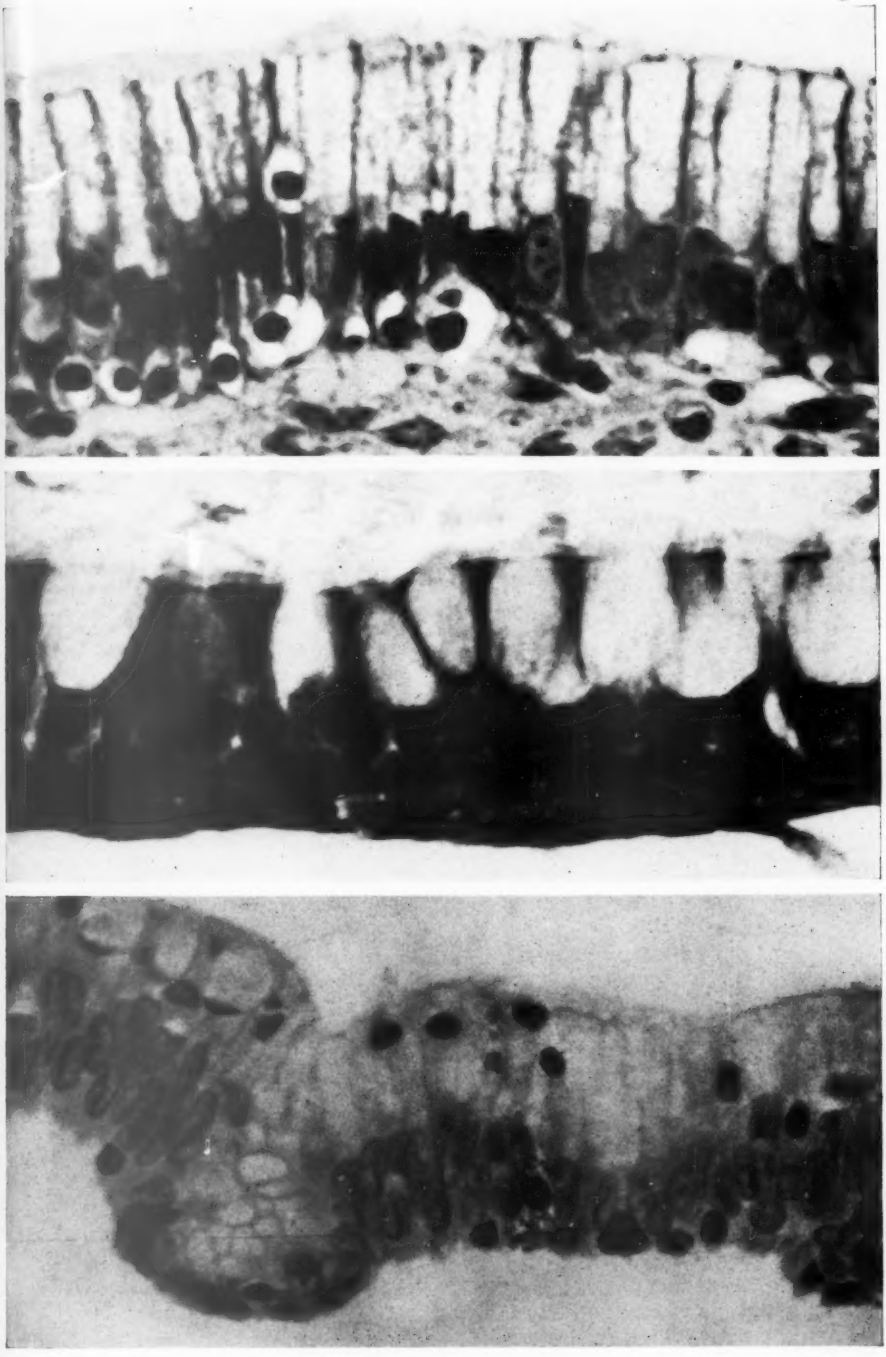
DESCRIPTION OF PLATES

Plate 105

Fig. 1. Lining epithelium of a multilocular cystadenoma, showing variations in phase of merocrine secretion. Some nuclei are compressed between adjacent cells; others have a supranuclear vacuole. There is longitudinal grooving of several nuclei. Of note are the basal nuclear masses. Terminal bars close the intercellular clefts in the free border. Heidenhain's iron hematoxylin stain. × 1065.

Fig. 2. Lining epithelium of a multilocular cystadenoma, made up of goblet cells, several of which are in merocrine excretion. A striate border is present. Heidenhain's iron-hematoxylin stain. × 1065.

Fig. 3. Lining epithelium of a parvilocular cystadenoma, showing two argentaffin cells. Of note is the nuclear infolding. Three goblet cells lie high in the epithelial layer. Masson's stain. × 1120.

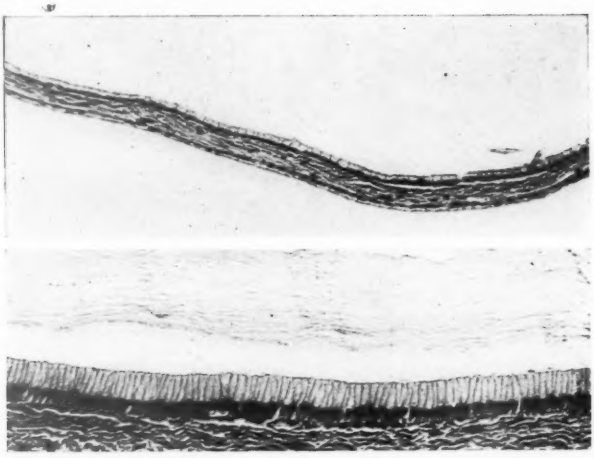


Reagan Pseudomucinous Cystadenoma

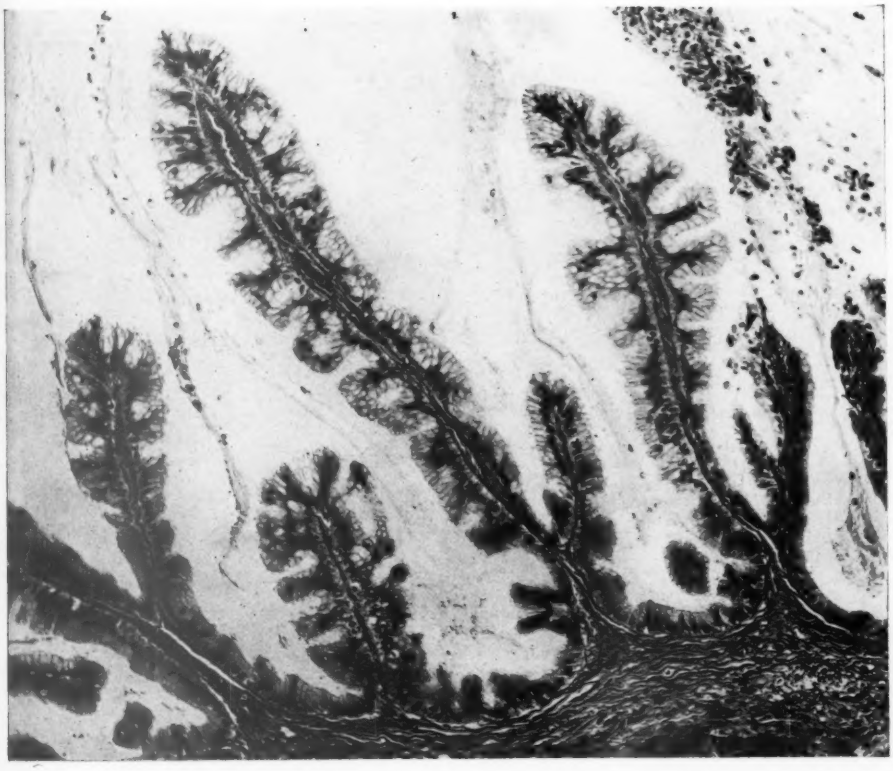
PLATE 106

FIG. 4. The flat epithelium lining the septum, and the columnar epithelium beneath are from the same locule. There is variation in height, even in the same locule. Hematoxylin and eosin stain. × 117.

FIG. 5. Cystadenoma of papilliferous type. Of note are the goblet cells over the papillae. Hematoxylin and eosin stain. × 770.



4



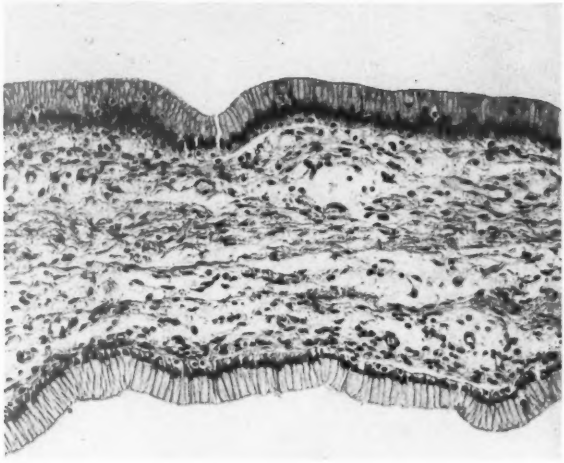
Reagan Pseudomucinous Cystadenoma

PLATE 107

FIG. 6. Epithelium lining septum of multilocular cystadenoma. Goblet cells lie beneath the free border of the upper epithelial layer. The lower epithelial layer shows the so-called ground glass appearance of the cytoplasm. Hematoxylin and eosin stain. × 177.

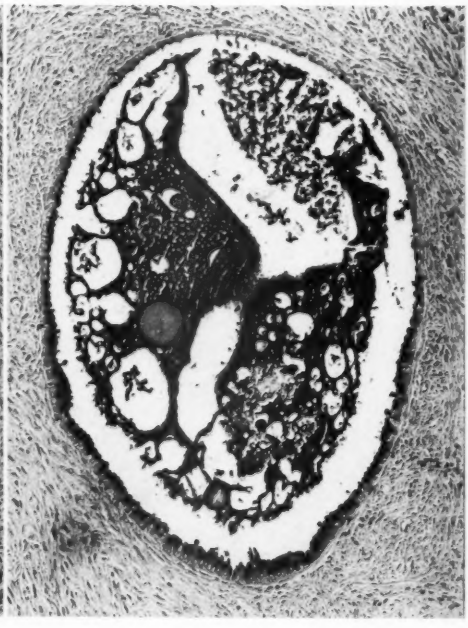
FIG. 7. Primitive microscopic locule from a small cystadenoma. Of note is the gradual transition from flat to columnar epithelium. Hematoxylin and eosin stain. × 150.

FIG. 8. Same primitive locule as seen in Figure 7. The columnar epithelium contains abundant secretion while the flat and cuboidal cells contain little or no secretion. Mucicarmine stain. × 150.



6

7



8

Reagan Pseudomucinous Cystadenoma

EXPERIMENTAL INFARCTION OF BONE AND MARROW*

JOSEPH H. BRAGDON, M.D.,† LEE FOSTER, M.D., and MERRILL C. SOSMAN, M.D.
(From the Departments of Pathology and Radiology of the Harvard Medical School and the Radiology Department of the Peter Bent Brigham Hospital, Boston, Mass.)

The long bones of mammals receive their blood supply from three sources. These are: (1) multiple small periosteal vessels penetrating and supplying the outer cortex; (2) epiphyseal vessels entering the ends of bones through ligamentous and capsular attachments; and (3) one (or more) nutrient arteries entering the shaft through nutrient foramina and supplying the diaphyseal marrow and inner cortex. There is considerable evidence that these vessels anastomose freely, particularly in the adult following disappearance of the epiphyseal cartilage plate, and it is for this reason that the possibility of bone or marrow infarction is often denied. Haslhofer,[1] writing on the circulation in bone and marrow in the Henke-Lubarsch Handbuch, stated that because of the richness of these anastomoses, disruption of even the largest source, *i.e.*, the nutrient artery, produces no sequelae, even in youth.

Clinicians, on the other hand, at times invoke infarction to explain otherwise cryptic bone lesions. Axhausen and Bergmann,[2] writing in the same volume with Haslhofer, presented clinical instances of aseptic bone necrosis which they ascribed to interruption of local blood supply. Phemister and his associates[3,4] have published radiographic and pathologic descriptions of lesions which they considered the result of marrow infarction. Some of the lesions occurred in patients who had previously had caisson disease.

Because the production of bone and marrow infarcts in animals has generally been considered impossible by conventional means, earlier investigators resorted to extensive stripping procedures or to the production of multiple small emboli designed to occlude large numbers of capillaries. Brunschwig,[5] attempting to produce infarction of the marrow of the femur in dogs, stripped the entire periosteum and simultaneously cut the nutrient artery. Despite this extensive trauma, he found no evidence of infarction in 15 adult dogs. In one young animal followed for 60 days, he described fibrosis and cyst formation within the marrow cavity. Among injection experiments may be mentioned those of Wollenberg,[6] who injected talc into the femoral artery of dogs and observed areas of necrosis in metaphyses and epiphyses. Bergmann,[7] on the other hand, following the injection of particles of silver suspended in gum arabic, found no changes in the epiphyses but widespread necrosis of

* Received for publication, July 23, 1948.
† Now at the National Institutes of Health, Bethesda 14, Md.

cortical bone. Kistler,[8-10] having failed to observe infarcts following simple ligation of the nutrient artery of the femur in rabbits, finally resorted to injecting suspensions of charcoal in acacia and masses of agglutinated bacteria. He injected these directly into the nutrient artery under pressures which were not measured but which were admittedly high. By these means he was able to produce areas of necrosis in the center of the metaphyses.

Huggins and Wiege[11] were the first to report changes following disruption of the nutrient vessels alone. In both mature and immature rabbits, ligation of the nutrient vessels to the femur was followed in all instances by infarction of marrow. Although in a few cases there was some periosteal and endosteal reaction about the operative site, they found no evidence of infarction of bone.

In view of the non-physiologic means usually employed previously and because of the absence of uniformity in the results obtained, it was decided to attempt once more, under better controlled conditions, the experimental production of bone and marrow infarcts.

Methods

Large, but skeletally immature, rabbits weighing between 2.3 and 3.3 kg. were subjected to transection of the nutrient artery of the femur. Immature animals were used throughout because, as shown by Drinker, Drinker, and Lund,[12] so long as the epiphyseal cartilage plate remains, anastomoses between diaphyseal and epiphyseal vessels are at a minimum.

The animals were anesthetized with intraperitoneal injections of sodium pentobarbital, supplemented when necessary with ether inhalation. Using sterile technic, the nutrient artery was exposed and cut as close to the bone as possible. As one or two small veins usually accompany the artery, they were inevitably destroyed as well. A description of the gross anatomy of the blood supply to this bone may be found in Kistler's first paper.[8] The distribution of arteries within the bone and their relative size are illustrated in Figure 8 of this paper. Bleeding was minimal and as a rule no ligatures were necessary. The wound was closed with cotton sutures. In one case, because of faulty technic, the wound became infected; this animal was discarded. As our primary purpose was to follow the cytologic sequence of events after infarction of the bone and marrow, most animals were operated upon bilaterally.

The animals were sacrificed with chloroform or air embolism at intervals of from 24 hours to 6 months. The femurs were cleaned and roent-

genograms were taken. They were then sawed transversely through the shaft and longitudinally through the epiphyses into fragments not over 2 cm. in length. These were fixed in Zenker's fluid or formalin, decalcified in nitric acid, embedded in celloidin, sectioned and stained with hematoxylin and eosin and Mallory's aniline blue. A total of 25 rabbits comprised the basis for this report.

Results

In practically every femur of which the nutrient artery had been severed, microscopic examination of diaphyseal marrow revealed evidence of infarction. Lesions were most common at the junction of the middle and lower thirds of the shaft, although in the majority of cases there was patchy infarction throughout most of the diaphysis. In several instances the lesions extended into the metaphyses, but epiphyseal marrow was never involved. The infarcted areas frequently abutted upon and involved portions of cortical bone.

The sequence of events within the infarcted marrow was as follows: 24 hours after injury, swelling and diminished staining capacity of hematopoietic cells as well as hemorrhage of slight to moderate degrees were seen. At 48 hours the infarcted areas were outlined by the appearance at the periphery of neutrophils and mononuclear cells containing cellular débris and hemosiderin. By the fourth day lack of uniformity of fat droplets gave evidence of necrosis and rupture of fat cells. At 1 week (Fig. 1), although the larger lesions still showed complete necrosis at their centers, there was considerable cellularity at the periphery. Large numbers of eosinophils made their appearance. The larger fat droplets were being actively phagocytosed by multinucleated giant cells. There was some increase in vascularity in this zone, but it was not prominent.

At 2 weeks the center of the infarct consisted of a very loose fibrillar and granular matrix from which cellular débris had largely disappeared and in which most of the fat droplets had been phagocytosed (Fig. 2). About the periphery there was a well defined zone 1 to 2 mm. wide resembling a granuloma (Fig. 3). Large mononuclear and giant cells predominated, but there were also numerous lymphocytes, eosinophils, and occasional focal accumulations of neutrophils. Connective tissue stains showed no fibrosis. An interesting feature at this stage was the presence of shrunken and pyknotic megakaryocytes completely engulfed by giant cells, a phenomenon which indicates that these cells are unusually resistant to lytic enzymes.

By 3 weeks the lesions again became less well defined by reason of the disappearance of many of the inflammatory cells. During the third and fourth weeks the impression was gained that fat-laden macrophages gradually became indistinguishable from normal fat cells. They appeared to lose their stainable cytoplasm and to assume more uniformity in size. By the eighth week healing had advanced to the point that only careful scrutiny would permit recognition of the lesion. The fat-containing cells were still somewhat more variable in size, tending to be larger than those in the surrounding uninfarcted areas. Interspersed among them were macrophages containing a faintly yellow, globular to crystalline material (Figs. 6 and 7). This substance, often present in considerable quantities, was insoluble in most of the usual fat solvents. It was not doubly refractile, even in frozen sections of formalin-fixed material, and was not acid-fast. It stained blue with Nile blue sulfate, and, although unidentified, is probably a lipid.

By 6 months, the previously infarcted areas could be identified with certainty only by the presence in them of macrophages containing this material. These cells were interspersed among what appeared to be normal fat cells. Such areas were without hematopoietic activity. Their most noteworthy characteristic, however, was the complete absence of fibrous scarring.

One animal developed diarrhea, lost weight rapidly, and died 12 days following surgery. Because of starvation the normal marrow was depleted of fat, its place being occupied by engorged sinusoids. The areas of infarction were readily recognizable against this background by the continued presence in them of fat. Figure 4 illustrates a section of femur from this animal.

In most cases there was infarction of bone as well as marrow. It was very common to see small areas of necrotic bone along the inner margin of the cortex of the shaft. Such areas occurred only where a marrow infarct abutted on cortical bone. (Infarcted bone could be recognized as early as 48 hours following loss of blood supply because the bone cell nuclei stained less deeply. Within 1 week the area appeared completely acellular.) When the marrow infarcts extended to involve distal or proximal metaphyses, there was necrosis of bony trabeculae within the zone of provisional ossification. In one case, 2 weeks following injury, there was evidence of a disturbance in longitudinal growth sequences.

The infarcted bone elicited no specific reaction. When the area involved was small, as was usually the case, it was gradually resorbed and replaced. As all bones were still growing at the time of infarction,

remodeling sequences frequently gave the appearance of necrotic bone incorporated within the mid-zone of cortex. If, on the other hand, a small necrotic area, because of its location, was destined to disappear completely during remodeling, it appeared more resistant to resorption than adjacent viable bone. When the area of infarcted bone was large, as happened less frequently, new bone was rapidly laid down, by periosteum and/or endosteum, in such a manner as to buttress the necrotic, and presumably weaker, area (Figs. 5 and 6).

Because of the small size of most of the bone infarcts, roentgenographic interpretation usually was equivocal. In Figure 9, however, may be seen a definite zone of reaction about an area of necrotic cortical bone.

Summary and Conclusions

Cutting the nutrient artery to the femur of the growing rabbit almost invariably resulted in infarction. This confirms the work of Huggins and Wiege[11] but is contrary to the experience of others. Infarction was most extensive in the diaphyseal marrow, extending in some cases well into the metaphyses. Epiphyses were never involved. In most instances there were small areas of bone infarction as well. The latter usually were limited to the inner zone of the diaphyseal cortex. Based on a small number of cases in which infarction extended into the metaphyses, it appears that any interference with longitudinal growth sequences was temporary.

The tissue sequences in both marrow and bone up to 6 months following injury are reported. In the case of marrow infarcts, the most striking feature was the complete absence of a fibrous cicatrix. Fat droplets released from necrotic fat cells were taken up by large phagocytic cells, which in time appeared indistinguishable from normal fat cells. The area of marrow infarction eventually was recognizable only by the absence in it of hematopoiesis and by the prolonged presence of numerous macrophages containing an unidentified yellow material, presumably a lipid.

Small areas of infarcted bone excited no reaction other than their very slow replacement by viable bone, a process comparable to "creeping substitution" in bone grafts. Depending on the direction of remodeling processes, they either became incorporated within viable bone or became resorbed. Resorption appeared somewhat slower than in the case of viable bone. When the area of infarction was larger, there was a rapid overgrowth of new bone, designed apparently to buttress the structurally weaker area of necrosis.

Except for the work of Huggins and Wiege,[11] whose interpretation of marrow sequences is somewhat comparable to ours, it is difficult to reconcile our results with those of other authors. Perhaps Kistler,[8-10] who has performed the most extensive work in this field and who failed to detect any changes following ligation of the nutrient artery alone, relied too much on roentgenographic evidence of damage. Brunschwig,[5] who did produce infarction of both marrow and inner cortex (by completely stripping the periosteum as well as cutting the nutrient artery of the femur in young dogs), reported fibrosis and cyst formation in the marrow. This is completely inconsistent with our results. Such results occurred in only one case, and unfortunately he published no photographs.

The human lesions described by Phemister and his associates[3,4] and ascribed by him to marrow infarction, also differ significantly from our experimental lesions. Roentgenographic and pathologic examination in his cases revealed in the femoral marrow widespread areas of liquefaction necrosis surrounded by a zone of calcification. This lack of similarity to our experimental lesions might be explained by differences in bone age at the time of injury, by differences in the duration of the lesions, or, more likely, by the much larger area of infarction permissible in human marrow. Size of infarcts has much to do with the manner in which they heal, or fail to heal.

Comparisons with osteochondritis dissecans and Perthe's disease, syndromes which have frequently been ascribed to infarction, are not pertinent, since in our rabbits there was presumably no interference with the blood supply to the epiphyses. Other examples of aseptic bone necrosis in the human may or may not be comparable.

REFERENCES

1. Haslhofer, L. Kreislaufstörungen des Knochens. In: Henke, F., and Lubarsch, O. (eds.) Handbuch der speziellen pathologischen Anatomie und Histologie. J. Springer, Berlin, 1937, 9, Pt. 3, 87–117.

2. Axhausen, G., and Bergmann, E. Die Ernährungsunterbrechungen am Knochen. In: Henke, F., and Lubarsch, O. (eds.) Handbuch der speziellen pathologischen Anatomie und Histologie. J. Springer, Berlin, 1937, 9, Pt. 3, 118–203.

3. Phemister, D. B. Changes in bones and joints resulting from interruption of circulation. *Arch. Surg.*, 1940, 41, 436–472; 1455–1482.

4. Kahlstrom, S. C., Burton, C. C., and Phemister, D. B. Aseptic necrosis of bone. *Surg., Gynec. & Obst.*, 1939, 68, 129–146; 631–641.

5. Brunschwig, A. Experimental infarction of bone marrow. *Proc. Soc. Exper. Biol. & Med.*, 1929–30, 27, 1049–1051.

6. Wollenberg. (Cited by Axhausen, G. Die aseptische Knochennecrose und ihre Bedeutung für die Knochen- und Gelenkchirurgie. *Acta chir. Scandinav.*, 1926, **60**, 369–396.)

7. Bergmann, E. Theoretisches, Klinisches und Experimentelles zur Frage der aseptischen Knochennekrosen. *Deutsche Ztschr. f. Chir.*, 1927, **206**, 12–87.

8. Kistler, G. H. Sequences of experimental infarction of the femur in rabbits. *Arch. Surg.*, 1934, **29**, 589–611.

9. Kistler, G. H. Sequences of experimental bacterial infarction of the femur in rabbits. *Surg., Gynec. & Obst.*, 1935, **60**, 913–925.

10. Kistler, G. H. Effects of circulatory disturbances on the structure and healing of bone. *Arch. Surg.*, 1936, **33**, 225–247.

11. Huggins, C., and Wiege, E. The effect on the bone marrow of disruption of the nutrient artery and vein. *Ann. Surg.*, 1939, **110**, 940–947.

12. Drinker, C. K., Drinker, K. R., and Lund, C. C. The circulation in the mammalian bone-marrow. *Am. J. Physiol.*, 1922, **62**, 1–92.

[*Illustrations follow*]

DESCRIPTION OF PLATES

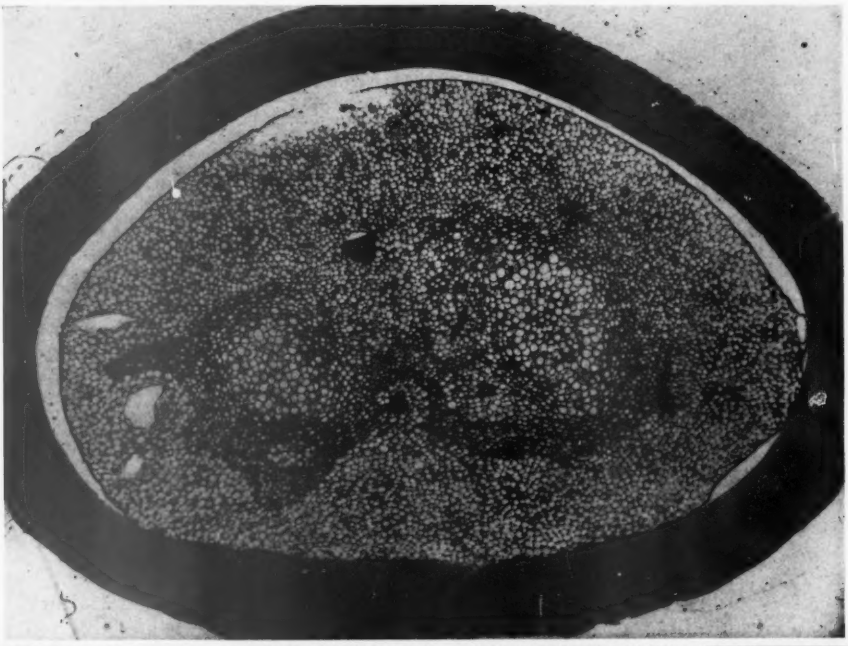
PLATE 108

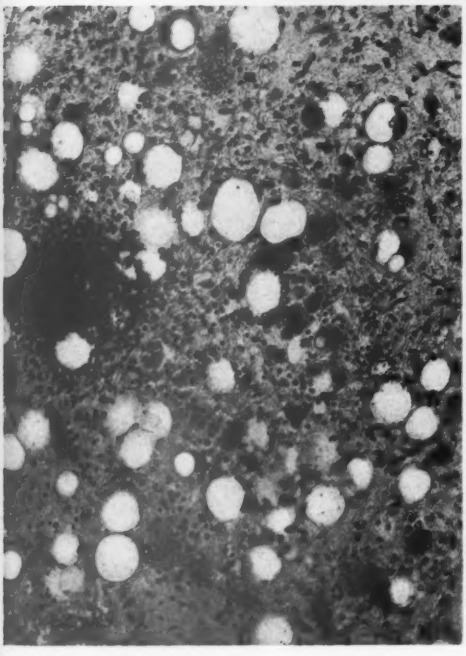
FIG. 1. Rabbit 191. Section through the junction of the middle and lower thirds of the shaft of a femur. The nutrient artery was cut 1 week prior to sacrifice. A large marrow infarct is well outlined by a zone of cellular reaction. × 29.

FIG. 2. Rabbit 190. Section of a marrow infarct of 2 weeks. Giant cells may be seen about fat droplets and engulfing pyknotic megakaryocytes. × 166.

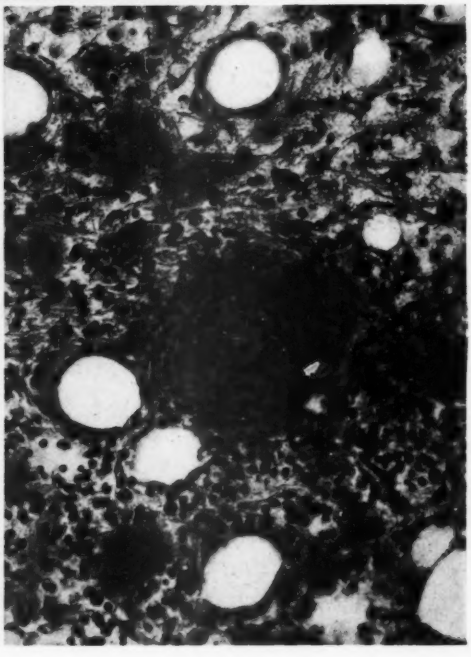
FIG. 3. Rabbit 189. Section through a zone of reaction about a marrow infarct of 2 weeks. There is a collection of large mononuclear cells. The larger fat droplets are surrounded by a layer of the same cells. × 332.

1





2



3

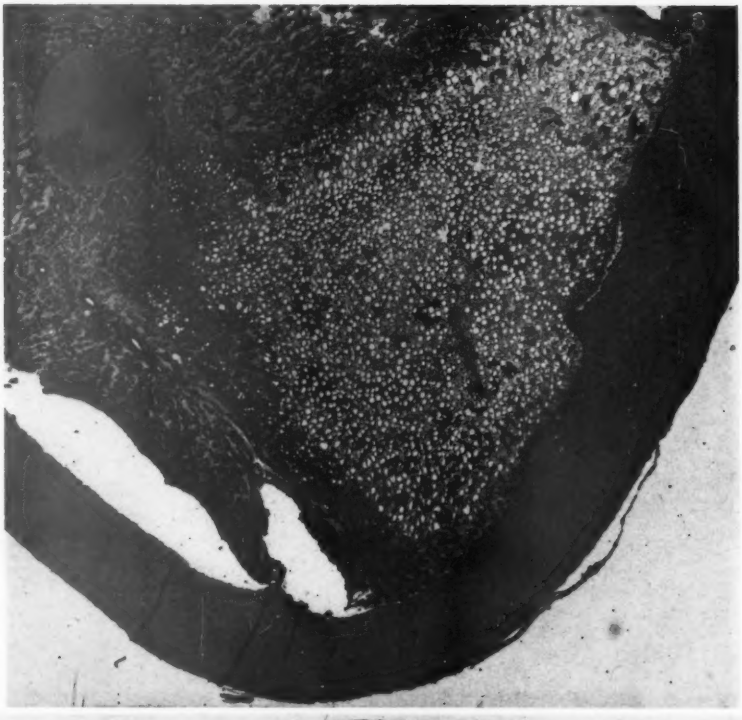
Bragdon, Foster, and Sosman

Experimental Infarction of Bone

PLATE 109

FIG. 4. Rabbit 180. Section of a marrow infarct of 12 days. This animal developed diarrhea, lost much weight, and died. In the uninfarcted marrow the fat has been mobilized and replaced by engorged sinusoids. Within the infarcted area fat droplets have been retained. Small areas of necrotic cortex may be seen along the margin of the marrow infarct. × 32.

FIG. 5. Rabbit 183. Section of marrow and cortical infarcts of 4 weeks. The zone of reaction about the marrow infarct has largely disappeared. An area of necrotic cortex has been buttressed by local overgrowth of new endosteal bone, forming an "internal splint." × 32.



4

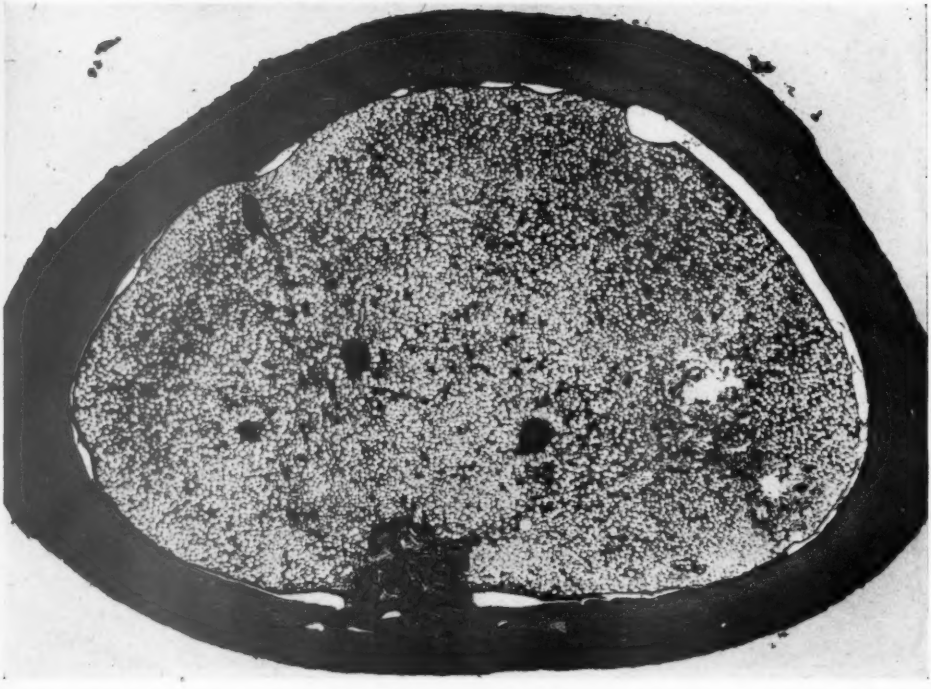


PLATE 110

FIG. 6. Rabbit 157. Section of marrow and bone infarcts of 4 months. The marrow infarct is now recognizable only by the presence of macrophages filled with an unidentified yellow material and by the absence of hematopoiesis. There is no fibrous cicatrix. The infarcted area of cortex has been buttressed by an overgrowth of new bone from the periosteal surface. × 31.

FIG. 7. Rabbit 157. Same section as shown in Figure 6. Portions of necrotic cortex still remain after 4 months. There is no evidence of active bone formation or destruction. A small field of infarcted marrow in the lower right corner shows macrophages containing an unidentified opaque yellow material. × 135.

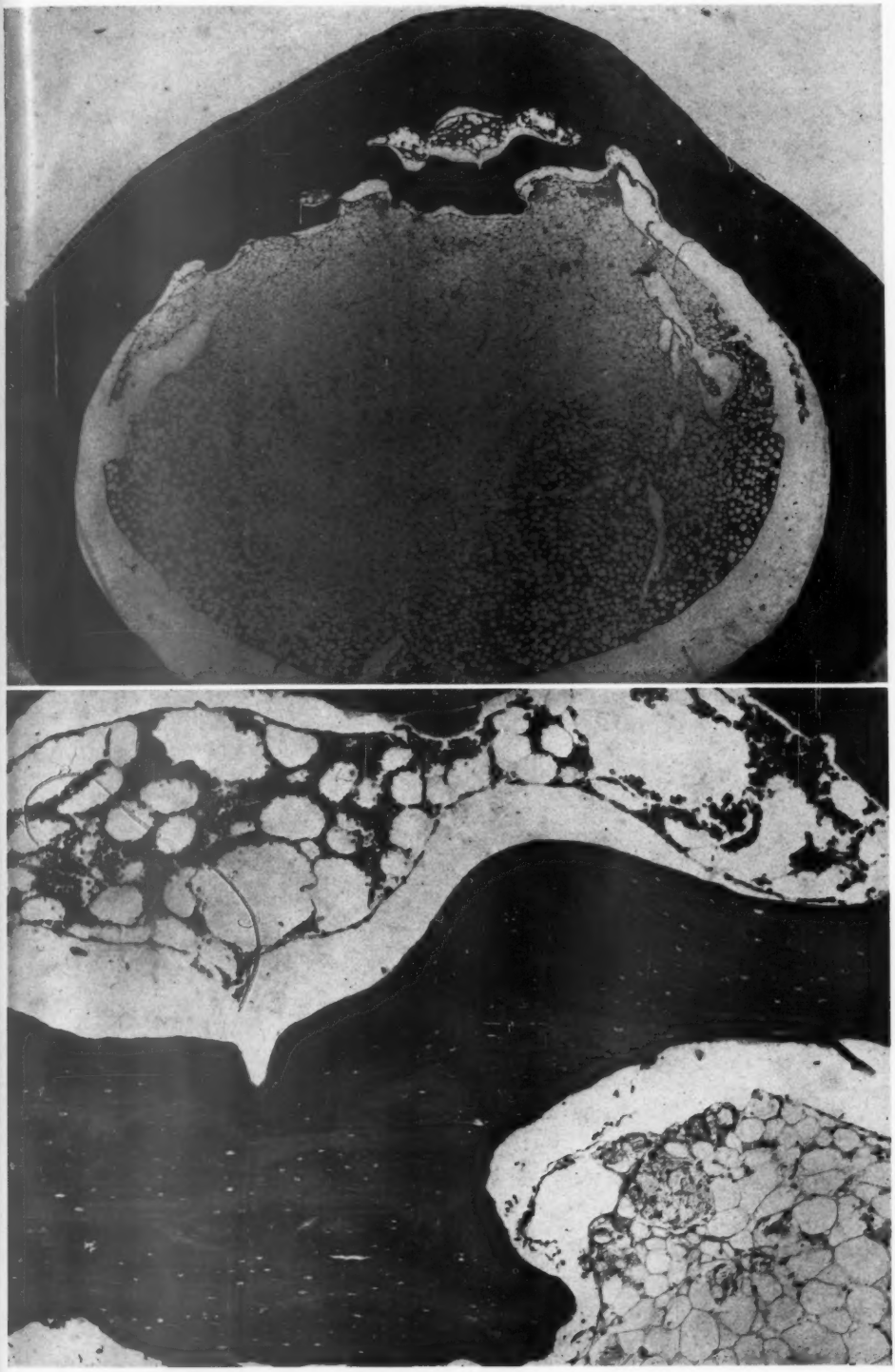
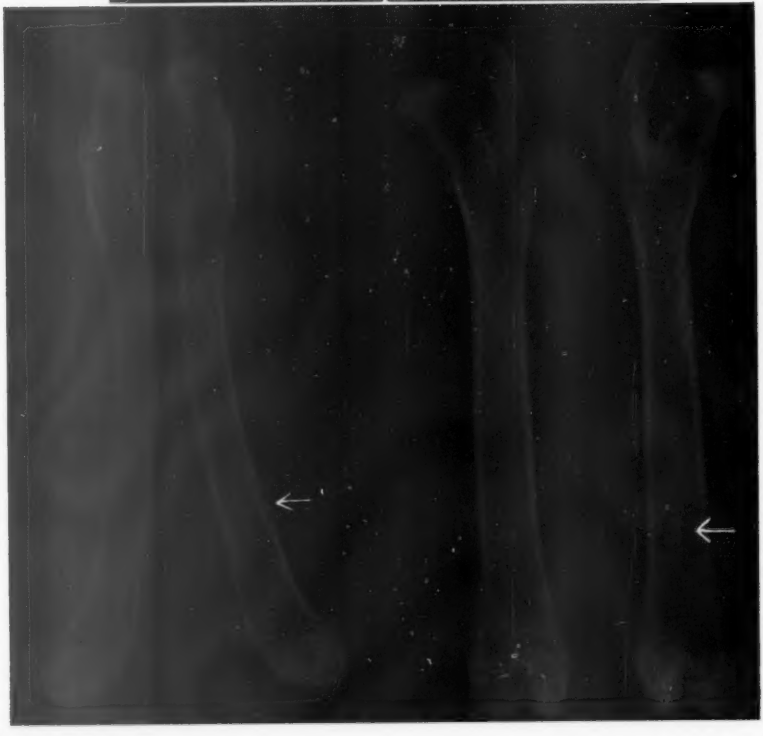


PLATE III

FIG. 8. Anteroposterior and lateral roentgenograms of the femur of a normal, immature rabbit following injection, via the abdominal aorta, of a radio-opaque fluid. The bone has been cleaned and partially decalcified. The small periosteal vessels did not fill, but several epiphyseal arteries, as well as the large nutrient artery and its branches, are delineated.

FIG. 9. Rabbit 157. Anteroposterior and lateral roentgenograms of both femurs 4 months after cutting the nutrient arteries and showing on the right an area of necrotic cortex buttressed by overgrowth of subperiosteal bone. (For comparison with Figures 6 and 7.)

8



9

THE TOPOGRAPHIC PATHOLOGY OF THE CEREBRAL LESIONS OF ACUTE POLIOMYELITIS IN MAN

I. The Medulla Oblongata*

James H. Peers, M.D., and R. D. Lillie, M.D.

(From the Pathology Laboratory, National Institutes of Health, Bethesda, Md.)

Our purpose in undertaking this study was to determine in detail the topography of the cerebral lesions of acute poliomyelitis based upon the systematic examination of a sufficiently large number of specimens to furnish a true average picture of the pattern of the disease, and to place the peculiarities of individual cases in proper perspective. It is recognized that this plan is not highly original. During the past several decades there have been numerous reports on the pathology of poliomyelitis. Many of these are based on one or a few cases, examined with varying thoroughness. The relatively few more detailed studies fall generally into two groups. One presents rather comprehensive conclusions based upon examination of random sections from a considerable number of cases. In the other the authors have subjected one or two specimens to a fairly intensive examination, generally directed toward confirming some one theory of pathogenesis. We have encountered only two reports in which a substantial number of brains were examined systematically and the results reported in adequate detail. One is the careful and elaborate investigation of Horányi-Hechst[1] based upon 24 complete central nervous system specimens from the 1931–1933 outbreaks of poliomyelitis in Budapest. The other is a description by Swan[2] of 6 brains from South Australia in 1937. Although only one lateral half of each brain was sectioned, the results are presented in considerable detail. Where possible we shall compare the findings of these two authors with our own in the discussion to follow.

For reasons of convenience we have arbitrarily divided this study into three sections: lesions in the medulla oblongata, described in this paper; lesions in the pons and cerebellum, and lesions in the midbrain, basal ganglia, and cerebrum to be discussed in two subsequent publications.

Material

Our results are based upon the examination of 42 specimens of brain. Of these, 6 were collected in Chicago in 1936, and 10 from the same city in 1937. From Detroit 8 were obtained in 1939 and 7 in 1941. The re-

* Investigation supported in part by a grant from the National Foundation for Infantile Paralysis, Inc.

Received for publication, August 12, 1948.

maining 11 were obtained singly or in small groups from random localities in the eastern part of the United States during 1941.

Clinical histories of varying degrees of completeness were available for 35 of the 42 specimens examined. For the remaining 7 specimens no clinical information of any sort was obtained. Twenty-four of the 35 patients were known to be males and 6 females. A reason for the large preponderance of males was not apparent. Practically two-thirds of the patients presented poliomyelitis of a pure bulbar type and died without the appearance of paralysis of the limbs. The remaining one-third were considered to be of mixed bulbar and spinal type. Most of these were admitted with bulbar symptoms, and toward the end developed some degree of paralysis of the limbs, usually of the arms. In only a few was there initially severe limb paralysis followed by bulbar collapse. The patients ranged in age from 1 to 33 years, but almost all were under 20. Of the 35 patients with known histories, 13 were in the first decade of life, 20 were in the second decade, and only 2 in the third decade. The duration of the disease, based upon the stated dates of onset of symptoms and of death, averaged 5 days for the bulbar type and 8 days for the mixed bulbar and spinal type. The extremes are presented by one patient with poliomyelitis of the fulminating bulbar type who died apparently within 24 hours after initial symptoms, and another with spinal, followed by bulbar, involvement who succumbed 20 days after the onset of paralysis of the limbs.

Technical Methods

All specimens of brain were fixed in 10 per cent formalin for several weeks or more, preferably and usually without previous sectioning, since the anatomic pattern rather than the cytology of poliomyelitis was the objective of the study. After sufficient hardening, the brain stem and cerebellum were separated from the hemispheres by a transverse incision through the cerebral peduncles just rostral to the anterior colliculi. The hemispheres were separated from one another by a longitudinal incision through the midline of the corpus callosum and the infundibulum. Then both hemispheres and the brain stem and cerebellum were sliced transversely at right angles to their axis at a series of predetermined levels. Blocks for embedding were prepared from each slice and marked with a code number identifying level and side of each. In all, 97 large blocks were so prepared from as nearly identical areas as possible from each specimen of brain. The location of these standard blocks will be indicated in the description of the lesions found in each brain area. Supplemental blocks occasionally were added to the standard series in order to display certain structures more satisfactorily.

After trimming and marking the blocks they were hardened for 3 days in 3 per cent aqueous potassium bichromate solution, washed overnight in running water, and embedded in paraffin. Sections 8 μ thick were stained routinely by the azure-eosin method of Lillie[3] which gave very satisfactory survey preparations. These were supplemented occasionally by special stains such as those for myelin and reticulum. Each slide was examined completely by means of a mechanical stage, and the types and locations of lesions were recorded.

For the examination of the medulla, sections were prepared routinely from four blocks cut respectively through the decussation of the pyramids, through the medulla at the level of the closure of the fourth ventricle, through the mid-portion of the inferior olives, and through the anterior end of the medulla at the emergence of the eighth nerve roots.

Observations

In the following description the various nuclei and cell groups are arranged in accordance with the generally accepted embryologic theory of nerve components. Those belonging to the somatic motor component are described first, followed in order by those of the branchial and visceral motor components, and the visceral and somatic sensory components. The medial and spinal vestibular nuclei, representing the special sensory component in the medulla, will be discussed subsequently with related structures in the pons, although for completeness lesions in the part of these nuclei appearing in our most rostral block are indicated in Text-Figure 4.

In describing the cellular changes in this series of specimens we have recognized and recorded lesions of three sorts. The first and most widespread is an infiltration of small lymphoid cells and some mononuclear cells in the Virchow-Robin spaces, forming perivascular collars of varying prominence. These are represented by small circles in the accompanying anatomic diagrams (Text-Figures 1 to 4). The second is a proliferation and accumulation of microglial cells in the ground substance, sometimes diffuse but most commonly in nodular aggregations of varying compactness. These are represented by groups of dots on the diagrams. Some of the smaller dense foci undoubtedly mark the site of destroyed nerve cells, but many of the larger, more diffuse groups appear to be a response to the injurious action of the virus on the ground substance independent of nerve cell destruction. The third lesion, necrosis of nerve cells, is symbolized by small crosses on the diagrams. These necrotic cells appeared as somewhat shrunken oxyphilic masses often lying in a vacuole. Occasionally such dead cells were undergoing neuron-

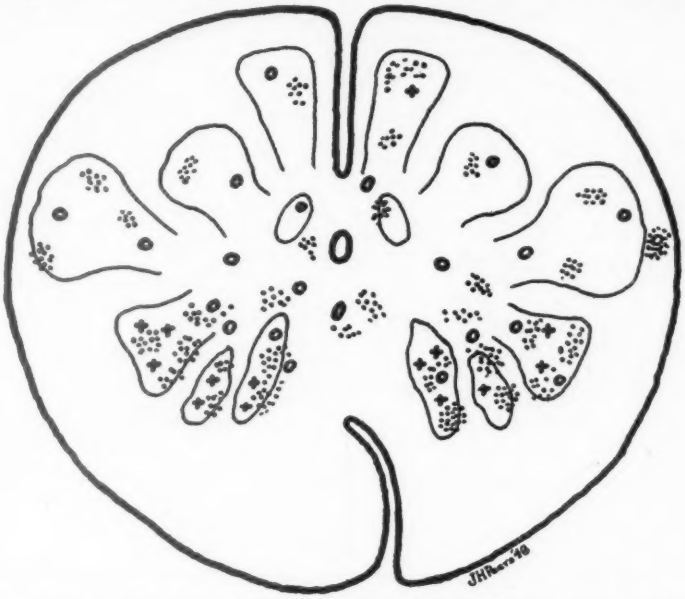
ophagia by phagocytic cells which in most specimens were mononuclear cells of microglial origin. In only a few specimens were polymorphonuclear leukocytes clearly identifiable among the reacting cells. In general neuronophagia was a much less prominent feature in the medulla than in the spinal cord, and many dead cells lay in a spongy ground substance among diffusely infiltrating microglia without exerting any evident chemotactic activity. Lesser degenerative changes such as partial chromatolysis and nuclear eccentricity were not recorded, partly because of the varying state of preservation of our specimens, and partly because of the difficulty of assessing the significance of such minor morphologic variations in nerve cells other than those of large somatic motor type. Only a rough estimate of the amount of cell loss was attempted in large-celled nuclei having a fairly regular and constant structure.

The *nucleus supraspinalis,* the most median of the groups forming the anterior horn column of large somatic motor cells, contains the cells of origin of the first cervical nerve supplying the small deep muscles of the upper part of the neck. Moderately severe lesions were present in this cell group in every specimen examined, irrespective of the duration of the disease or the outbreak from which the specimen was obtained (Text-Fig. 1). Perivascular round-celled infiltration and focal and diffuse interstitial infiltration were moderately abundant. It was estimated roughly that from one-half to two-thirds of the nerve cells had disappeared. In the great majority of the specimens a few necrotic cell bodies were still to be seen, occasionally undergoing active neuronophagia, chiefly by phagocytic microglia. In a single specimen of approximately 11 days' duration both cells and ground substance of the entire horn on both sides had been destroyed. The resulting small cavities were crowded with phagocytic microglia filled with lipoid débris, forming foam cell abscesses.

The nucleus of the *spinal accessory* nerve forms a small discontinuous strand of nerve cells of motor type, lateral to the supraspinal nucleus and extending upward from about the sixth cervical segment for a short distance into the lowest level of the medulla. Its cells supply the motor innervation for the sternomastoid muscle and a large part of the trapezius muscle. By some, the nucleus is considered to form the most caudal group of the branchial motor column. Lesions in this nucleus were moderately severe in all specimens and corresponded closely to those in the immediately adjacent nucleus supraspinalis (Text-Fig. 1). Perivascular and interstitial infiltration was prominent. Occasional necrotic cell bodies were seen in almost all specimens but the degree of cell loss

could not be estimated fairly because of the small and variable size of this nucleus in the medulla.

The *hypoglossal nucleus* is a well defined group of typical large nerve cells of motor type, located just beneath the floor of the fourth ventricle. These cells furnish the motor innervation of the muscles of the tongue. Damage was of about equal degree at the level of the ventricular closure

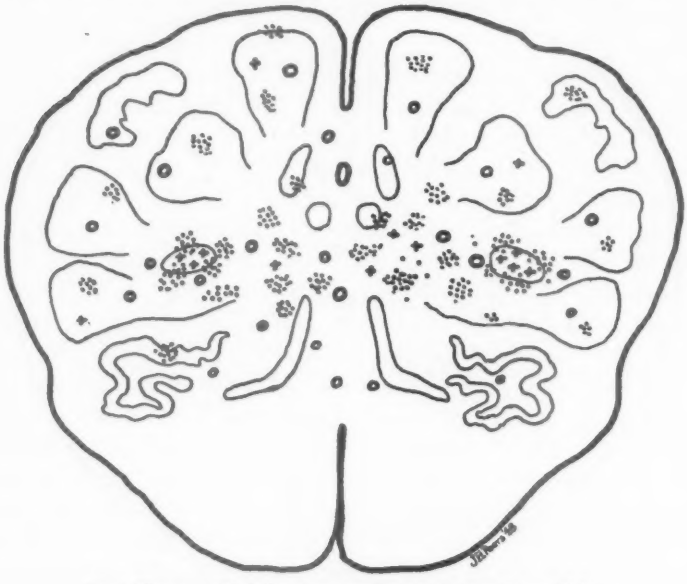


Text–Figure 1. Medulla at decussation of pyramids. In this and subsequent figures, perivascular infiltrations are represented by circles, interstitial infiltrations by groups of dots, and dead cells by crosses. Major nuclear groups are indicated in thin outline.

(Text-Fig. 2) and at the olives (Text-Fig. 3), but was considerably less severe than might have been expected in a nucleus of pure somatic motor neurone type. Some degree of perivascular infiltration, usually sparse, was present in nearly all specimens. Focal microgliosis, also generally of mild degree, was present in about 80 per cent of the sections. On the other hand, necrotic nerve cells were few and were noted in only one-third of the specimens. Such destructive lesions usually were unilateral, and the opposite nucleus often appeared practically normal. Occasionally a small foam cell abscess occupied a portion of the affected nucleus. The observed lesions rarely appeared to have been sufficiently extensive to have caused recognizable paresis of the tongue during life, a fact also noted in clinical descriptions of poliomyelitis. Horányi-Hechst[1] re-

ported a similar light and irregular damage in this motor nucleus. Swan[2] evidently observed greater damage in his small series, since he stated that the ambiguus and hypoglossal nuclei were equally affected.

The *nucleus ambiguus* is a long, irregular column of large nerve cells of motor type lying in the lateral part of the reticular substance and more or less recognizable throughout the length of the medulla. It forms

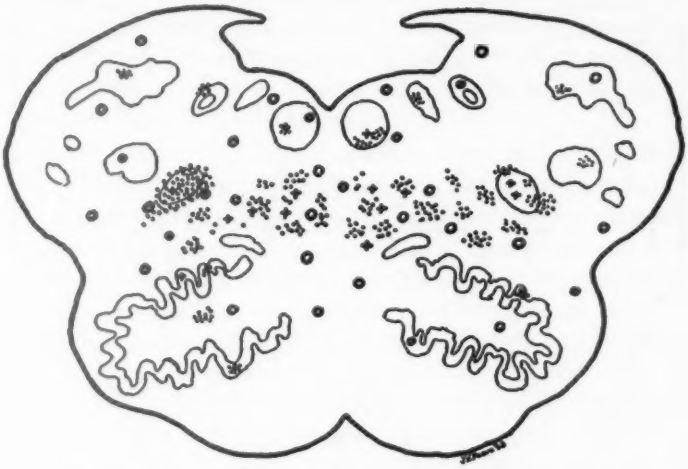


Text-Figure 2. Medulla at the closure of the fourth ventricle.

part of the branchial motor component. Axones from its cells run in the glossopharyngeal, vagus, and accessory nerves to supply the striated musculature of the pharynx and larynx. The nucleus ambiguus was invariably damaged and usually severely in all specimens at all levels (Text-Figs. 1, 2, and 3), as would be expected from the pharyngeal paralysis and obstruction of the airway so common in the terminal stages of fatal poliomyelitis. The severity of the infiltrative lesions appeared to be of about equal degree at the three levels examined. The lesion was graded as moderate in about one-third and severe in two-thirds of the specimens. Necrotic nerve cells were seen in about one-half of the specimens. They were noted somewhat more frequently at the level of the pyramidal decussation, quite likely because at this level the nucleus ambiguus is most poorly defined and other necrotic cells of the upper end of the anterior horns were incorrectly assigned to it. The severity

of the lesions was roughly equal on the two sides in about three-fourths of the specimens, and notably asymmetric in the remaining one-fourth.

At the level of the mid-portion of the olives (Text-Fig. 3), where the nucleus ambiguus is largest and most clearly defined, there was almost complete destruction of one or both nuclei in one-fourth of the specimens. Not only had almost all the nerve cells disappeared, but the ground substance had dissolved and the resulting cavity was filled with

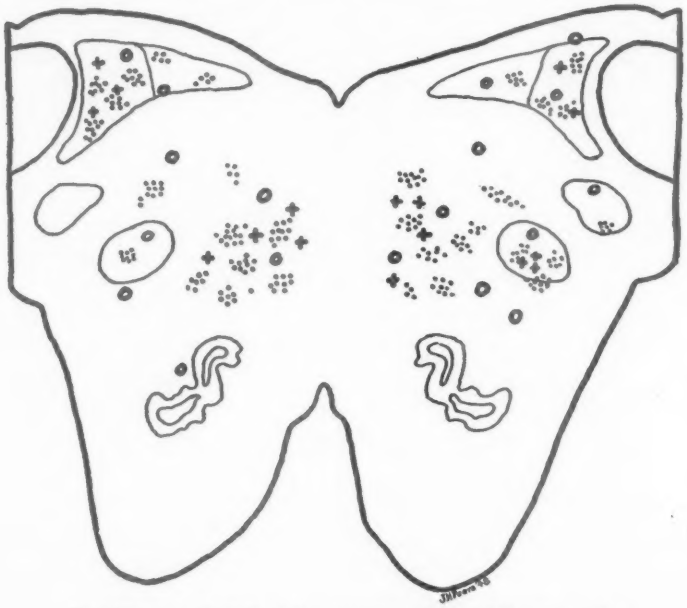


Text-Figure 3. Medulla through the mid-portion of the inferior olives.

a mass of phagocytic microglia with vacuolated cytoplasm filled with lipoid débris. Such foam cell abscesses were unilateral in 6 and bilateral in 4 specimens. Their occurrence could not be correlated with the reported duration of the illness, or the epidemic from which the specimen was obtained.

The *motor nucleus of the facial nerve,* also part of the branchial motor component, is an oval group of large cells of motor type lying on the border between the pons and medulla, in a ventrolateral position corresponding to that of the more caudal nucleus ambiguus. Its cells send motor fibers to all of the small muscles of the face, the stylohyoid and the posterior belly of the digastric muscle. In our material the facial nuclei never entirely escaped injury (Text-Fig. 4). There was some degree of perivascular infiltration, usually scanty, in all specimens. Foci of microgliosis were present in all but 2 specimens. When divided according to the abundance of this interstitial reaction, one-third were recorded as mild, one-third moderate, and the remaining one-third as marked.

One or two necrotic nerve cells were observed in one-half the specimens. In an additional one-fourth, dead cells were moderately numerous. In the remaining one-fourth no cells were recorded as actually necrotic. In the majority of cases the lesions seemed roughly symmetric on the two sides, but occasionally one nucleus was severely injured while the opposite presented only minimal inflammatory reaction. Both Horányi-Hechst[1] and Swan[2] recorded briefly essentially similar observations.



Text-Figure 4. Medulla at the emergence of the eighth nerve roots.

There was no satisfactory correlation between the amount of damage observed in the facial nuclei in sections and the reported occurrence of facial paralysis in the available clinical histories. Sections from patients stated to have had facial paralysis showed severe histologic lesions in the facial nuclei, often on the corresponding side, but many other specimens presented apparently equally severe lesions although the available history made no mention of facial paralysis. In only one instance did the section from a patient having facial paralysis during life fail to present significant lesions in the facial nuclei. This excess of lesions in the facial nuclei over clinically observed facial paralysis may be due in part to the likelihood that lesser degrees of facial paresis passed unnoticed in the critically ill or moribund patients. No lesion observed was so extensive as totally to destroy the nuclei in section, and

in most instances there probably were sufficient functioning neurones left to prevent marked or complete facial paralysis.

The *reticular substance* is an irregularly shaped mass of gray matter in the center of the medulla, filling the interspaces between the large fiber tracts and discrete nuclei. It extends through the entire length of the medulla. It is composed of small and medium-sized nerve cells among which are a number of large cells of motor type, especially in the more anterior portion. This formation appears to be a relay center for many reflexes tending to maintain the body in static position. Vomiting and swallowing reflexes are also mediated through it. Of especial interest is the fact that the seat of control of the rhythm of respiration appears to be located in its more caudal portion.

As might be expected in a group of fatal cases of poliomyelitis, chiefly of the bulbar type, the reticular substance presented marked lesions in all specimens and at all levels (Text-Figs. 1 to 4). Both perivascular and interstitial infiltration were pronounced. The severity of the damage increased notably in the more anterior levels near the pons, and the necrotic nerve cells were most numerous in the anterior giant-celled portion having connections with the vestibular nuclei. In addition, comparison with control sections of normal brain suggested that up to one-half of the large cells had disappeared without leaving any trace behind. Both Horányi-Hechst[1] and Swan[2] reported essentially the same lesions in their specimens. Correlation of these lesions with signs and symptoms in the living patient was virtually impossible. The majority of patients on admission were critically ill and severely prostrated and static positional reflexes for obvious reasons were not examined and recorded. The majority of patients died of respiratory failure, but most often the available records did not distinguish between paralysis of the muscles of respiration and collapse of the central regulatory mechanism. In five cases, however, the records mentioned jerky and irregular respiration with pauses and marked variation in rate, and in all of these patients lesions were pronounced in the posterior end of the reticular substance.

The *dorsal motor nucleus of the vagus,* principal representative of the visceral motor component in the medulla, is a long slender group of small spindle-shaped cells lying beneath the floor of the fourth ventricle somewhat lateral to the mid-line. Axones from its cells pass through the vagus nerve to the ganglia of the thoracic and abdominal sympathetic plexuses. There they synapse with secondary neurones innervating the heart and a wide variety of smooth muscle and glands in the respiratory and digestive tracts. It is thus the central efferent nucleus of the widely distributed autonomic nervous system. Unlike the peripheral

portions of the sympathetic system which rarely show lesions in poliomyelitis, this central nucleus frequently exhibited some inflammatory reaction, although such changes were seldom marked. One or two perivascular collars of lymphoid cells were observed in about 80 per cent of specimens at all three levels, but it is doubtful how many of these represented reaction to toxic material originating in deeper structures and oozing out along emergent vessels traversing the nucleus. In about one-half of the specimens definite parenchymatous lesions in the form of small foci of microgliosis were present at the level of the pyramidal decussation (Text-Fig. 1) and at the ventricular closure (Text-Fig. 2). At the level of the inferior olives, where the nuclei are larger and more distinct (Text-Fig. 3), such focal lesions were seen in only one-third of the specimens. No necrotic nerve cells were noted in any specimen at any level. Both Horányi-Hechst[1] and Swan[2] made brief mention of slight to moderate involvement of the dorsal vagus nuclei in about one-third of their specimens.

The *nucleus of the tractus solitarius*, visceral sensory component in the medulla, is an eccentric mantle of gray matter wrapped around the rostral end of the descending tractus solitarius much like cotton about an applicator stick. It consists of small nerve cells of sensory type on which terminate the incoming fibers of the facial, glossopharygeal, and vagus nerves. The anterior part is concerned with the sense of taste while more caudal portions are part of the reflex mechanisms of swallowing, respiration, and vomiting. At the level of the ventricular closure, lesions were uniformly mild but frequent. Scanty perivascular infiltration was noted in almost all specimens, while slight focal microgliosis was present in slightly over two-thirds. No necrotic cells were seen. In 2 specimens one nucleus was intact and in one both nuclei were free of lesions at this level.

More anteriorly, at the level of the olives (Text-Fig. 3), damage in the nucleus solitarius was somewhat less frequent and noticeably more uneven in severity. In 5 specimens both nuclei were intact, and in 7 more, one nucleus was free of lesions. In the remainder, slightly over two-thirds of the series, there was both perivascular infiltration and focal microgliosis. Usually this was of moderate degree, but in 3 cases it was recorded as marked and in one, single necrotic nerve cells were seen. It is worthy of note that in the three specimens showing severe lesions in the solitarius, the dorsal motor vagus and the ventral motor (ambiguus) nuclei were also severely damaged. However, the converse was not true and severe injury was commonly noted in the ventral motor vagal (ambiguus) nuclei unaccompanied by correspondingly marked lesions

in the nucleus solitarius. This nucleus is not specifically mentioned by either Horányi-Hechst[1] or Swan.[2]

The *nucleus of the spinal tract of the trigeminal nerve* forms a rather large bilateral mass of small and some medium-sized nerve cells scattered sparsely in a fine-grained fibrillar matrix. This nucleus, a continuation of the substantia gelatinosa capping the posterior horns, extends through the medulla to the mid-pons, but at higher levels it is less distinctly marked out. It and the remaining nuclei described below form the somatic sensory component of the medulla. Clinically apparent alterations in its function as first nucleus of the sensory portion of the trigeminal nerve was hardly to be expected. However, as sensory nucleus for the mucous membranes of the mouth and nose, lesions in it are of considerable interest in relation to these regions as possible portals of entry of the virus.

At the most caudal of the three levels studied—that through the pyramidal decussation (Text-Fig. 1)—at least a mild degree of perivascular infiltration appeared in all but one specimen. In the absence of parenchymatous lesions in the nuclei, the significance of these is uncertain since many of the vessels supply deeper and more severely damaged regions of the medulla and traverse the rather bulky spinal trigeminal nucleus in reaching the meningeal surface. Hence the milder degree of perivascular infiltration may be only the cellular reaction to effusion of toxic products of degeneration from the severely damaged deep structures. Parenchymatous lesions in the form of diffuse and focal microglial proliferations were noted in the spinal trigeminal nuclei of both sides in about one-half the specimens. Usually they were of unequal severity. In one-fourth of the specimens such lesions appeared on only one side—perhaps accidentally, chiefly on the right. In the remaining one-fourth no parenchymatous lesions were seen in sections at this level. In the large majority of specimens with lesions there was only mild microglial proliferation with one or two small focal accumulations. The majority, although not all, of these lesions were located in the inner or spongy zone of the nucleus. In about 10 per cent of the specimens focal and diffuse microgliosis was quite marked and occurred not only in the gray matter of the nucleus but also in irregular, usually perivascular patches in the white matter of the descending spinal tract of the trigeminal nerve lying external to the nucleus. Very rarely a single necrotic cell was seen.

At the level of the ventricular closure (Text-Fig. 2), parenchymatous lesions in the spinal trigeminal nuclei were almost identical in frequency and severity with those at the level of the pyramidal decussation.

The upper portion of the spinal trigeminal nucleus appearing at the

level of the olives (Text-Fig. 3) presented considerably less damage than was noted at the lower levels. Parenchymatous lesions were only about one-half as frequent. One-third of the specimens showed a few small foci of microgliosis on both sides; in one-sixth, lesions were unilateral, while the remaining one-half presented no parenchymatous lesions. Only 2 specimens ran clearly counter to the general trend for damage to diminish rostrally. In both of these, lesions in deeper structures—ambiguus and reticular substance—were unusually severe at this level. In practically all specimens there was some degree of perivascular round-celled infiltration, although it was usually somewhat less marked than at lower levels in spite of the usually severe and extensive lesions in the deeper structures. Swan[2] made note of the occasional occurrence of focal infiltrations in the spinal trigeminal nucleus, but saw no necrotic nerve cells, probably because of the limited number of specimens available to him. In contrast, Horányi-Hechst[1] stated categorically that this nucleus remained entirely free of histologic alterations in every case. No explanation for this discrepancy is apparent.

The *nucleus gracilis* is the first relay station in the long dorsal column pathway. Axones from the spinal ganglia of the lumbosacral segments bearing proprioceptive sensation from the lower limbs ascend in the funiculus gracilis and end on the cells of the nucleus gracilis. Axones of the cells of the nucleus gracilis cross to the opposite side in the sensory decussation and ascend in the medial lemniscus to the ventral nuclei of the thalamus. In about 80 per cent of the specimens some degree of perivascular infiltration, usually bilateral and of mild degree, was present (Text-Figs. 1 and 2). Definite local damage in the form of focal and diffuse microgliosis was somewhat less frequent. About three-fourths of the specimens showed such lesions. Of these, somewhat less than one-half showed only one or two small foci on one side only. In the remainder, scattered foci of reactive microglia were present on both sides. In about 20 per cent of the specimens, one or two necrotic nerve cells were noted in addition to the inflammatory infiltration. In only 3 specimens, or about 7 per cent of the total series, was the nucleus gracilis found to be entirely free of inflammatory reaction.

The *nucleus cuneatus* is very similar in structure and connections to the nucleus gracilis, but its incoming fibers have their origin in the ganglia of the upper thoracic and cervical segments, and carry proprioceptive sensation from the arms and the neck. The axones of its cells run upward along with those from the nucleus gracilis in the medial lemniscus and end in the ventral thalamic nuclei. In the series of specimens as a whole, lesions in this nucleus were identical in number and

intensity with those in the nucleus gracilis (Text-Figs. 1 and 2). In individual specimens also, the lesions were almost always of nearly equal degree in the two nuclei. In no case was one nucleus spared when the other was severely damaged. Both Horányi-Hechst[1] and Swan[2] appear to have observed a corresponding amount of damage in these nuclei of the long dorsal tracts.

The *lateral cuneate nucleus,* in spite of its name and location, is not a part of the cuneate fasciculus and nucleus. Rather it is the analog in the medulla of Clarke's column in the cord. To its cells are brought tone impulses from muscle, joint, and tendon receptors in the neck and possibly in the upper limb. Axones from cells of the lateral cuneate nucleus pass via the restiform body to the vermis, especially to its anterior part. At the level of the ventricular closure the lateral cuneate nuclei present some degree of perivascular infiltration—usually scanty, and bilateral in about three-fourths of the specimens (Text-Fig. 2). Interstitial focal microgliosis was present in about 40 per cent of the whole series. In one-third of the specimens showing parenchymatous lesions, the focal infiltrations were few and present on only one side. In slightly more than one-fifth the nucleus appeared entirely normal at this level.

Farther rostral, at the level of the olives (Text-Fig. 3), the lateral cuneate nuclei presented more frequent and somewhat more severe damage. In all but 2 specimens examined there was some degree of perivascular infiltration, generally small in amount and almost always bilateral. Interstitial microgliosis was noted in about one-half the specimens. Unilateral and bilateral parenchymatous lesions were about equally frequent. Single necrotic nerve cells in one nucleus or the other were observed in about 15 per cent of the specimens examined. In only two instances were there no visible lesions. Horányi-Hechst [1] and Swan [2] do not specifically mention the lateral cuneate nuclei.

The *inferior olives* form a large and conspicuous nuclear mass extending through most of the length of the medulla. The olives are sensory nuclei by embryologic derivation and function, being a relay station, en route from the brain stem and cervical cord to the cerebellum, for impulses coordinating movements of the eyes, head, and upper limbs. The course of the afferent fibers to the olives is not known with certainty, but the axones of the olivary cells cross the midline to join the restiform body and to be distributed widely to the cerebellar cortex of the opposite side. Those from the superior and medial accessory olives go to the older midline portion of the cerebellar cortex, while those from the ventrolateral part of the main nucleus have been traced to the more recently developed cerebellar hemispheres. A few fibers are believed to descend

in the olive-spinal tracts of Helweg to the anterior horn cells of the upper segments of the cervical cord.

As was the case with the large sensory nuclei previously described, perivascular round-celled infiltration of slight to moderate degree was observed in the inferior olives of practically every specimen in the series. Only 2 were noted as being entirely free of lesions of any sort, and in each case the olives were normal only in the caudal section through the closure of the ventricle. A number of these perivascular infiltrations doubtless did not represent damage in the olivary nuclei but rather reaction to drainage of toxic products along radiating vessels from the overlying severely injured reticular substance.

However, parenchymatous lesions indicating direct injury to the olivary nuclei were observed in 27 per cent of the specimens. They occurred with nearly equal frequency in sections through the middle of the olives (Text-Fig. 3) and in the posterior part of the nuclei at the level of the ventricular closure (Text-Fig. 2). For the most part the lesion consisted of single loose foci of proliferating microglia, almost always on one side only. Rarely, single foci were found on both sides. Many of these foci of microgliosis seemed to represent response to a direct injurious action of the virus on the ground substance rather than proliferation secondary to degeneration and necrosis of a nerve cell. The foci were rather large and loose. The regular pattern of nerve cells in the olivary nucleus was not disturbed and nearby nerve cells showed no morphologic evidence of degeneration. One such focus was found among the white fibers of the hilum of the olive where no nerve cells are present. However, in 6 specimens there was actual necrosis of one or two nerve cells, with neuronophagia in the form of small, tight clusters of microglia in addition to the larger and loose interstitial foci not visibly related to nerve cell degeneration. Horányi-Hechst[1] briefly noted the occurrence of slight lesions in the olives in single cases in his material. Swan[2] reported the frequent occurrence of perivascular infiltration in the hilum, but saw no parenchymatous lesions in the olives in the limited number of specimens in his series.

The spatial arrangement of these parenchymatous lesions in the contour of the olivary nucleus was unexpected and perhaps significant. If the virus had entered the olivary nucleus by simple seepage from the overlying severely damaged nucleus ambiguus and reticular substance, it would have been expected that lesions would have tended to be concentrated in the dorsal portion of the olive nearest the source of the virus. If the virus penetrated the side of certain axones of the olivary cells as they traversed the restiform body, a random distribution of

lesions throughout the olivary nuclei would have seemed most likely. In fact, neither pattern of lesions appeared in the specimens. Instead, the glial foci were quite clearly concentrated in the medial half of the olivary nucleus, and about equally distributed between the dorsal and ventral plates of the nuclear complex. This medial portion of the olive is connected with the midline areas of the cerebellum which is the only portion of the cerebellum where lesions are found regularly. Such a concentration of lesions in the two interconnected portions of what otherwise appear to be internally homogeneous brain areas is noteworthy.

Summary and Conclusions

In a study of the anatomic distribution of the lesions of acute poliomyelitis in 42 specimens of the medulla oblongata it was found that lesions of maximum severity are regularly encountered in the reticular substance, nucleus ambiguus, and supraspinal and spinal accessory nuclei. Other somatic and branchial motor nuclei unaccountably present much less severe and constant lesions.

Nuclei of the sensory components frequently contain a few small lesions. Such lesions are somewhat more numerous and severe in the somatic than in the visceral sensory nuclei. The occurrence of single necrotic nerve cells with typical reaction in each of these nuclei in a few specimens indicates that the cells of the sensory nuclei are not completely refractory to the virus of poliomyelitis.

While a few individual specimens presented moderate quantitative differences from the average pattern of lesions, the series as a whole gives a general impression of uniformity. Within the series no grouping is discernible that suggests an essential difference of pathogenicity of supposedly various strains of virus. Also, there are no consistent differences in disease pattern corresponding to the stated duration of illness, or to whether it began with bulbar or spinal symptoms. We are obliged to conclude that death in poliomyelitis occurs when a certain and fairly constant amount of damage is produced in the medulla, and that the final pathologic end-result, in the medulla at least, is relatively uniform, and apparently independent of the route of spread of the virus, or of the speed with which the vital centers are overwhelmed and disorganized.

References

1. Horányi-Hechst, B. Zur Histopathologie der menschlichen Poliomyelitis acuta anterior. *Deutsche Ztschr. f. Nervenh.*, 1935, 137, 1–54.
2. Swan, C. The anatomical distribution and character of the lesions of poliomyelitis. *Australian J. Exper. Biol. & M. Sc.*, 1939, 17, 345–364.
3. Lillie, R. D. Histopathologic Technic. The Blakiston Co., Philadelphia & Toronto, 1948, p. 83.

THE RECOVERY OF SKELETAL MUSCLE FIBERS FROM ACUTE ISCHEMIA AS DETERMINED BY HISTOLOGIC AND CHEMICAL METHODS*

JOHN W. HARMAN, M.B.,† and RODNEY P. GWINN, M.D.

(From the Department of Pathology, University of Wisconsin School of Medicine, Madison, Wis.)

In a previous study of the histologic changes induced in skeletal muscle by acute ischemia,[1] it was found that death of muscle fibers occurred in an orderly, progressive manner up to 12 hours, at which time the extent of necrosis is considerable. Subsequently it was shown[2] that, even following brief intervals of ischemia, further extension of the necrosis was not prevented when a vascular occlusion was released. The duration of ischemia was perpetuated by both stasis and capillary damage, thereby increasing significantly the extent of necrosis. Although the increase in necrotic fibers was marked, many appeared normal by histologic standards after as long as 6 hours of complete ischemia. The fate of such intact fibers is of paramount importance; their number and their ability to regain contractility, among other factors, may determine the degree of functional recovery of the muscle as a whole.

It has been tacitly assumed[3,4] that, with the initiation of ischemia in skeletal muscle, there is a *pari passu* depletion of energy reserves, onset of autolysis, and incidence of death in all the muscle fibers. Depletion of energy reserves is presumed to be a criterion of cell death[5] and in some instances of autolysis.[6] While current studies of chemical events appear to substantiate such a view, the behavior of the histologic pattern is not so easily reconciled with it, unless there are within many fibers alterations more subtle than are detectable by microscopic examination. Because of this discrepancy it is relevant to ascertain the ability of such fibers to recover from ischemia and to determine the factors responsible for cell death or irreversible changes in fibers; it is particularly important to decide whether the essential irreversible event is structural or chemical. If chemical alteration alone is the determinant, the histologic picture may be equivocal in the evaluation of viability; whereas if the histologic structure represents a reliable criterion of viability, its exact significance deserves closer consideration. This relationship of histologic structure to viability and chemical change is, therefore, the object of the present study.

* Read by title at the Forty-Fifth Annual Meeting of The American Association of Pathologists and Bacteriologists, Philadelphia, March 12 and 13, 1948.

Received for publication, July 15, 1948.

† Denis J. Coffey Traveling Fellow in Physiology, University College, Dublin (National University of Ireland).

Experimental Procedures

Ischemia was produced in one hind extremity of male albino rabbits, weighing 2000 to 3000 gm. and of Sprague-Dawley male rats, weighing 250 to 300 gm., by a technic previously described.[2] After varied periods of ischemia the tourniquet was released and the extensor muscles were excised during nembutal narcosis from both the ischemic and normal legs, following recovery for 0, 3, and 24 hours. Immediately before excision the muscles were tested for contractibility by faradic current.[1] The excised muscles were fixed in 10 per cent formalin, at 37° C., embedded in paraffin, and sectioned 8 μ thick. The muscles were sampled to allow representative examination of all parts; sections were stained with hematoxylin and eosin. In each section the normal and necrotic fibers were identified, and 100 consecutive fibers were counted differentially, so that an average percentage of normal and of diseased fibers was calculated for each muscle. The contralateral muscle from the normal limb served as a control in each animal.

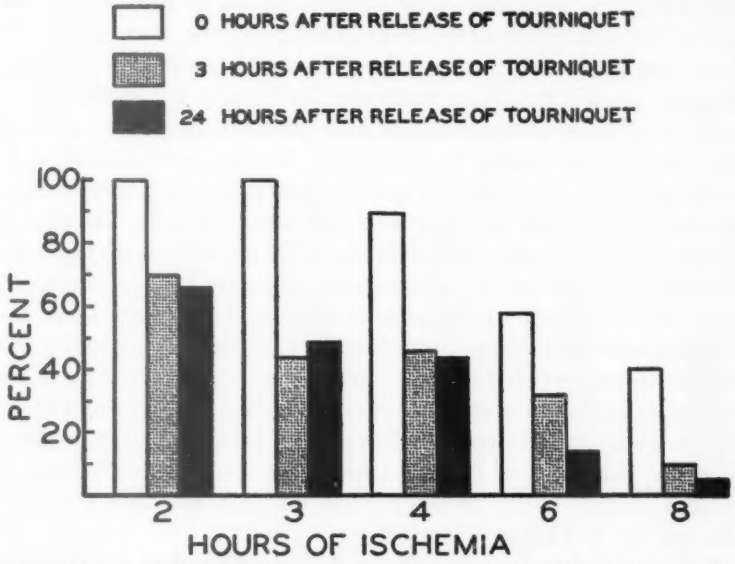
In a second series of male rats the tourniquet was applied to one thigh for 4 hours and a recovery period of 24 hours permitted, after which the animals were narcotized with nembutal and the extensor muscles of the legs exposed. The distal tendinous attachment of the tibialis anticus muscle was carefully sectioned and the muscle freed by gentle dissection to its proximal fleshy attachment. Contractility was tested by a faradic current and sufficient time for restitution then allowed, after which the muscle was rapidly excised and plunged into liquid air. The frozen muscle was then pulverized in a chilled bronze chamber. The chamber consisted of a central well into which was fitted a closely adapted solid cylinder for crushing the tissue, with a surrounding moat for containing the chilling liquid, usually liquid air. Portions of crushed tissue were distributed to tared 50 cc. flasks containing 5 cc. of cooled 10 per cent trichloracetic acid and zinc sulfate solutions, respectively. After reweighing the flasks the tissues were examined for several components. The fraction contained in trichloracetic acid was exhaustively extracted and the extract analyzed quantitatively for adenosine triphosphate, phosphocreatine, and acid-soluble phosphorus by the scheme of LePage.[7] Glycogen was estimated on the extracted residue by the method of Good, Kramer, and Somogyi,[8] with determination of sugar by adaption of Hoffman's[9] technic to a spectrophotometric procedure. The fraction extracted by zinc sulfate solution was used for determination of lactic acid by Winnick's microdiffusion method.[10]

The analytic figures were corrected for increases in volume due to edema by simultaneous excision of the digitorum longus muscles from

the ischemic and normal legs. These muscles were weighed in tared crystal dishes at once and were reweighed after drying for 12 hours at 110° C. Corrected values might have been expressed by MacFarlane and Spooner's formula[11] or, as in this instance, interpreted directly for 100 gm. of dried tissue. It is assumed that there is no significant loss of tissue protein from the damaged muscle.

Results

With complete unrelieved ischemia the histologic pattern changed from the normal syncytoid[1] structure at the second hour, with disappearance of the normally conspicuous longitudinal striations and accentuation of the transverse striations, which were much less conspicuous in



Text-Figure 1. The percentage of histologically normal fibers after different periods of ischemia and intervals of release for 65 rabbits.

the rapidly fixed muscle. Further ischemia was associated with the appearance of Bowman's discoid degeneration (Fig. 1) at about the fourth hour; the number of fibers affected by this change reached a maximum of nearly 90 per cent by the twelfth hour of ischemia. If the ischemia was relieved at any time after the second hour, there was a further extension of degenerated fibers over the next 3 hours, when the number reached a maximum and did not increase even after 24 hours of recovery (Text-Fig. 1). It was apparent, however, that, in contradistinction to the muscles with unrelieved ischemia, those with release of

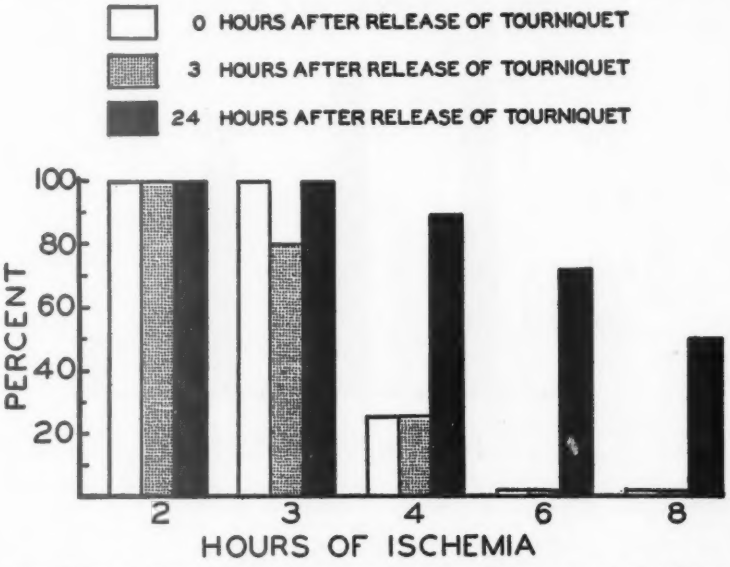
occlusion contained fibers with several types of necrosis. Many of the fibers conformed in appearance with Zenker's hyaline degeneration; they were large, homogeneous, and contained a structureless, faintly acidophilic cytoplasm (Fig. 2). Others were composed of a granular, disorganized, deeply acidophilic cytoplasm and might be designated as showing granular necrosis (Fig. 3) in accordance with Fishback's description.[12] The percentage of these different forms of necrosis in the muscles released from ischemia was variable and unpredictable; occasionally the Bowman's type was predominant, and at other times either the Zenker or the Fishback form was most prevalent. It was not possible to discern the transformation of one form to another, except that certain fibers appeared to progress through either the discoid or waxy degeneration to a granular disintegration. It was clear, however, that neither Zenker's degeneration nor Fishback's granular degeneration was to be seen in muscles with unrelieved ischemia; they occurred exclusively in ischemic muscles to which the blood supply was readmitted.

All three forms of degeneration were therefore grouped as necrotic fibers in the fiber counts, because of this independent variability. In Text-Figure 1 it is seen that not until the fourth hour did necrotic fibers appear in completely ischemic muscles; after that time the rate of appearance was progressive until at 8 hours of ischemia only 33 per cent of structurally normal fibers remained. When the tourniquet was released for a period of 3 hours prior to excision of the muscle there was a sudden increase in the necrotic fibers, which was proportional to the duration of ischemia and unrelated to the number seen before release of the obstruction. By the eighth hour of ischemia, only 10 per cent of the fibers were structurally normal (Fig. 4). The extension of the period of release up to 24 hours did not significantly alter the percentage of residual normal fibers, although some decrease was manifest after ischemia of 6 and 8 hours' duration.

It is apparent that release of the occlusion, rather than obviating the necrosis, favors its extension. The return of blood flow is associated with a sudden increase in necrotic fibers, which rapidly reach a maximum with little subsequent progression. The connection between the inflow of blood, the appearance of the two forms (Zenker's and Fishback's types) of degeneration, and the sudden increase in necrotic fibers seem correlative. These phenomena may well be, but in such an instance one would expect that after ischemia for 2 and 3 hours with subsequent release, such muscles would contain necrotic fibers solely of these types, especially as Bowman's disks are absent before release, yet all forms

are usually well represented. Even if the inflow of blood is instrumental in the increase of necrosis, other factors cannot be excluded.

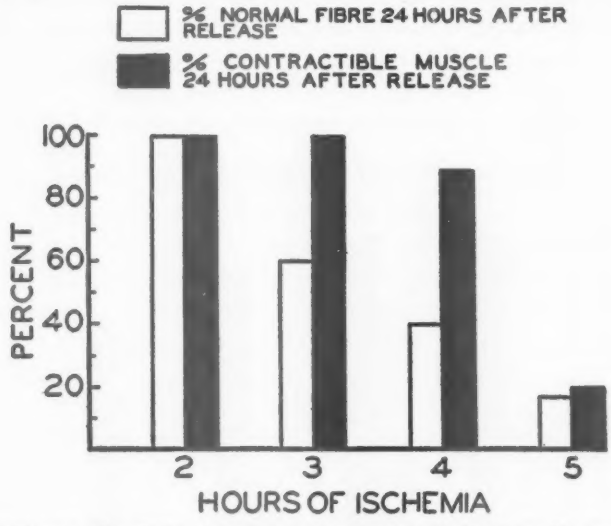
When the contractility of muscles was assessed after various periods of ischemia, it was found that nearly all had lost this property by 4 hours, and all had become non-contractible by six hours (Text-Fig. 2). By 3 hours after release of vascular occlusion the state of the muscle was little altered, whereas by 24 hours after release a considerable number of muscles had regained contractibility to greater or less degree. Up to 90 per cent had recovered marked ability to contract after 4 hours of ischemia, and 50 per cent were again contractible, at least in part, after 8 hours of ischemia. As might be expected from the greater percentage of surviving fibers after shorter durations of ischemia, the muscles sub-



Text-Figure 2. The percentage of contractible muscles after various periods of ischemia and intervals of release for 70 rabbits.

jected to briefer periods of ischemia recovered a greater strength of contraction. The survival of histologically normal fibers after different durations of ischemia was paralleled not only in the recovery of contractibility, but also in the vigor of the contraction, which bore an approximate proportion to the extent of fiber survival. In Text-Figure 3 it is demonstrated that the same relationship between contractibility of muscles and recovery of normal fibers also applies to ischemic rat muscle, which follows a similar trend.

To elucidate more exactly the interrelationship of fiber survival and ability of muscles to contract, the muscles were divided into the contractible and non-contractible groups. In each of these the percentage of histologically normal surviving muscle fibers was determined and the average assessed for each period of ischemia for both rabbit and rat muscle. The results are shown in Text-Figure 4. In all contractible muscles the normal fibers comprised a considerable part of the muscle mass, even after 6 hours of ischemia; the low level of 10 per cent was reached only after 8 hours of vascular occlusion. On the other hand, among the non-contractible muscles the average percentage of normal

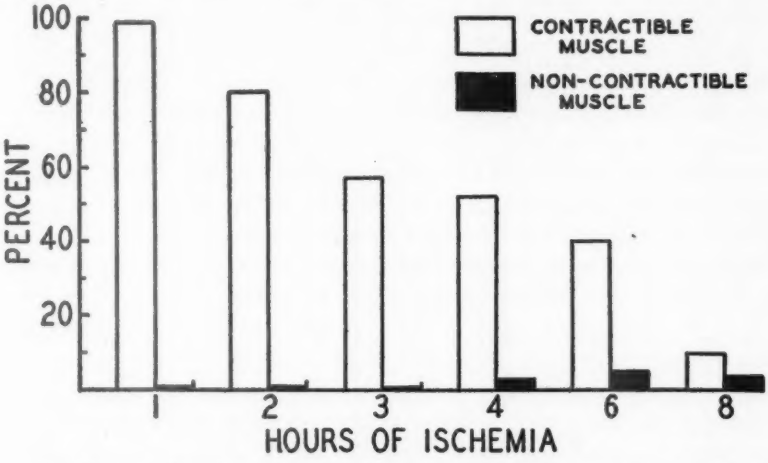


Text-Figure 3. Correlation of percentage of normal fibers and contractible muscles after different periods of ischemia and 24 hours of recovery in 60 rats.

fibers never exceeded 4 per cent; the majority of muscles in this group contained no normal fibers whatsoever. It again became evident that the ability to respond as well as the strength of response to electrical stimulation is intimately related to the presence and number of these histologically normal fibers.

The maximum degeneration of fibers was accomplished within 3 hours after release of the occlusion, whereas contractibility failed to return before about 24 hours subsequent to release despite the presence of many histologically normal fibers. In such instances there existed a form of structurally normal but physiologically inactive fiber. Chemical analyses on ischemic muscle from several rabbits and rats immediately after termination of 4 hours of ischemia revealed that there was

complete depletion of various energy reserves at such time. Such was the condition, also, after a further 3 hours of recovery. However, after a recovery period of at least 24 hours following an ischemia of 4 hours, the restitution of high energy compounds was considerable; adenosine triphosphate, glycogen, and phosphocreatine reaccumulated to between one-quarter and one-third of that found in the contralateral normal muscle (Table I). Whereas in normal muscle such might signify a marked depletion, in this ischemic contractible muscle in which the percentage of histologically normal fibers was 40 per cent or lower (Text-Fig. 3), the re-gain of essential metabolites fairly approximated that of the



Text-Figure 4. Correlation of contractibility and normal fiber percentage at 24 hours after release from different periods of ischemia in 65 rabbits and 60 rats.

surviving contractible tissue. The reaccumulation of these compounds essential to the contractility of muscle accorded well with the structural survival of muscle fibers. Although there was recovery of oxidative metabolic function and resynthesis of both phosphorylated compounds and glycogen, the injured muscles contained a significantly greater amount of lactic acid, indicating the presence of some glycolysis. It is true, however, that these muscles were heavily infiltrated with polymorphonuclear leukocytes to which such an accumulation of lactic acid might be due in part. There also was considerable stasis and sluggishness of circulation which might contribute to the slow removal of the products of contraction induced by the faradic test for contractibility.

The interval required for recovery adequate to permit contraction appears so markedly a function of time that the results of an additional

TABLE I

Results of Chemical Analyses on Ischemic Rat Muscle *

Animal no.	Tissue weight	Water	Acid-soluble phosphorus	Phospho-creatine	Adenosine triphosphate†	Lactic acid	Glycogen‡
	gm.	%	mg. %	mg. %	mg. %	mg. %	mg. %
1 Nor.	0.6579	69.6	136.8	243	321	19.5	430.6
1 Isc.	0.7726	82.1	31.2	72	63	23.8	37.6
2 Nor.	0.6212	74.7	123.4	236	296	16.9	414.2
2 Isc.	0.8064	82.2	29.6	62	79	39.5	48.1
3 Nor.	0.8785	71.3	117.5	194	326	20.1	386.7
3 Isc.	0.9729	79.6	28.4	41	120	27.6	56.2
4 Nor.	0.8853	62.4	128.7	233	341	17.8	448.2
4 Isc.	0.9945	89.9	32.4	56	96	37.6	16.2
5 Nor.	0.4064	58.9	121.4	221	352	18.7	426.1
5 Isc.	0.7751	71.2	37.5	49	102	28.4	61.0
6 Nor.	0.3359	67.8	156.0	254	301	22.4	451.0
6 Isc.	0.6383	79.0	22.9	59	51	29.7	
7 Nor.	0.4214	66.8	139.2	216	314	13.6	433.7
7 Isc.	0.5177	77.2	44.1	42	78	23.2	
8 Nor.	0.4133	66.0	119.5	201	329	17.4	424.6
8 Isc.	0.5989	76.5	16.8	21	91		47.2
9 Nor.	0.6081	67.2	124.2	214	311	18.5	419.5
9 Isc.	0.8449	74.6	26.7	68	84	29.6	29.2
10 Nor.	0.6544	75.8	136.6	229	324	22.0	401.2
10 Isc.	0.8216	80.1	31.2	36	81	28.7	102.1
11 Nor.	0.7569	66.3	134.0	231	317	20.6	443.2
11 Isc.	0.8455	77.2	39.7	44	76	34.2	57.1
12 Nor.	0.6423	67.1	127.2	217	312	23.1	432.0
12 Isc.	0.8735	75.4	41.2	31	106	31.2	42.1
Mean: Nor.			130.3	224.0	328.5	19.2	425.9
Isc.			31.0	48.4	85.6	30.3	53.4
Stand. dev.) Nor.			±10.6	±16.2	±17.2	±2.7	±18.2
Isc.			± 7.7	±14.5	±17.8	±4.9	±19.7
Stand. error) Nor.			± 2.2	± 3.3	± 3.5	±0.5	± 3.7
Isc.			± 1.5	± 2.9	± 3.6	±1.1	± 4.6

* All rats were subjected for 4 hours to total ischemia of one hind limb and then allowed to recover for a further 24 hours, at termination of which time the chemical analyses were performed on the tibialis anticus muscle. Prior to excision for chemical analysis, the ischemic muscles were tested faradically and were found to be contractible in all animals.

† Calculated from pentose and easily hydrolyzable phosphate (7 min. at 100° C. in HCl).

‡ Glycogen is expressed as glucose.

experiment designed and performed on 25 rats are pertinent. The animals, after 4 hours of ischemia and under nembutal narcosis, were prepared by exposure of the tibialis anticus muscle and attachment of the distal tendon of this muscle to the lever of a kymograph. Tests by direct faradic stimulus elicited no response until at least 21 hours had

elapsed subsequent to relief of ischemia. The factors responsible for this particular time interval of recuperation are not clarified by these experiments and further investigation along different paths will be necessary.

Discussion

The interpretation of structurally normal muscle fibers, which survive the period of anoxia, as viable cells is substantiated by the recovery of contractibility and resynthesis of energy reserves by muscles containing a significant number of such fibers. Moreover, the ability to contract and the degree of contraction are closely allied to the proportion of histologically normal fibers which persist. Further support of this is offered by the fact that in such muscles as become non-contractible, normal fibers are entirely lacking or present only in small numbers, whereas in contractible muscles these fibers comprise a major element of the tissue. From this it may be deduced that the structural condition of the fibers is an important factor in their ability to recover from ischemia. Fibers which maintain structural integrity throughout the period of vascular occlusion and the first few hours thereafter are with few exceptions capable of regaining function; it is tantamount to saying that structurally intact fibers are viable fibers, although in the early phases of recovery they contain no energy reserves and are still non-contractible.

The view that such fibers, which contain no energy reserves and are non-contractible, are still viable requires elucidation. Definitions of viability depend in one respect upon criteria which are consistent with non-viability or death. If the content of energy-rich compounds and the presence of irritability are accepted as decisive characteristics of living tissue, it might appear that their absence signifies death of the tissue. Upon closer scrutiny it is not entirely logical to conclude that because these attributes signify viability therefore their lack indicates death. It consequently becomes apparent that the state of non-viability and the state of simple depletion of energy reserves may not always be synonymous, although they are usually concomitant. All tissues which suffer exhaustion of high-energy supplies do not *ipso facto* die, even when the duration of depletion is prolonged. Many tissues, muscle particularly, recover their anabolic capacity and regain function. The decision of the incidence of death is related, therefore, not so much to the loss of essential metabolites and function as to the inability of the tissue to regain these within an appropriate length of time. The irreversibility of the loss is the essential factor rather than the loss itself.

In muscle the gap between loss of energy reserves and its irreversibil-

ity is very marked, and for many fibers may represent several hours. If such loss itself represents death or "biochemical death" as it has been called, then all fibers should die simultaneously about the time of exhaustion of these reserves and the cessation of expenditure of energy. We have found this to be untrue for muscle; the fibers commence to die at the time of complete exhaustion of the energy reserve and gradually are destroyed in proportion to the duration of ischemia. The state of the energy reserves, therefore, is indecisive as an indication of the condition of the muscle fibers. This is more accurately reflected in the structural picture of the fibers provided that adequate time is allowed for full development of necrosis, when the histologically normal and abnormal fibers may be accurately separated. Since the structure of the fiber is more closely related to its state of health than is the immediate energy content, it is probable that the decisive determinant of irreversibility is not the condition of energy expenditure but the structural integrity. Although it is acceptable to agree with Schoenheimer[13] that energy must be expended continuously to maintain structure against its tendency to collapse, it may be emphasized that the critical event in the tendency toward irreversibility is the collapse of structure rather than the preliminary breakdown of the energy cycle. The series of events leading to ischemic necrosis may then be enumerated as anoxia, glycolytic depletion of energy reserves, cessation of energy exchange, and structural collapse, which last decides the biochemical irreversibility.

It has been proposed by MacFarlane and Spooner,[11] on the basis of indirect evidence, that ischemic muscle fails to resynthesize phosphorylated adenosine compounds because it is unable to re-aminate inosinic acid. But we have found on direct chemical analysis a considerable resynthesis of adenosine triphosphate and phosphocreatine, as well as a marked ability to contract, in muscles recovering from ischemia. The reason for this discrepancy is not readily apparent. It is pertinent, however, that in intact muscle the inosinic acid formed during contraction is rapidly re-aminated,[14] and that this re-amination occurs only under aerobic conditions.[15] The relative anoxia induced by the stasis which follows protracted ischemia[2] may for a time prevent this re-amination, in view of its sensitivity to lack of oxygen. When stasis diminishes and the circulation improves, re-amination takes place. The evidence of our experiments substantiates this view and receives support from the observations of Bollman and Flock[3] that in instances in which fatal shock is avoided the return of acid-soluble phosphates to the ischemic muscle is appreciable after several days, and that after 2 weeks the animals regain functional activity of the ischemic leg.

The observation that following long periods of ischemia many fibers are capable of oxidative resynthesis finds its corollary in the work of Bielschowsky and Green,[16] who postulated that ischemic muscle might be capable of resynthesizing adenosine triphosphate and were able to achieve this *in vitro* with the enzyme systems of both ischemic and normal rat muscle. Our studies have confirmed their results *in vivo* and established the validity of their view that the re-accumulation of the phosphorylated compounds is mainly a matter of adequate recovery time. The enzyme system is present and intact so that other factors may be responsible for the slow rebuilding of the energy reserves. The nature of these factors is not clarified by the present experiments, although there is some indication from the persistent high level of lactic acid that important elements may be stasis and a high state of aerobic glycolysis.[17] The significance of stasis in the perpetuation of ischemia has been discussed in a previous study[2] and is further suggested in the present analysis by the occurrence of Bowman's discoid necrosis in muscles recovering from 2 to 3 hours of ischemia. These forms usually are not found before the fourth hour of ischemia, so that their occurrence in these muscles under the circumstances of recovery indicates some persistent ischemia. In view of the previous vascular experiments[2] the cause of the relative ischemia may be attributed to capillary stasis. It is probable that this persistent stasis and anoxia preclude the accumulation of biochemical reserves and account for the slow restitution of contractility.

Summary

The correlation of histologic structure, irritability, and biochemical alterations in skeletal muscles recovering from acute ischemia reveals that histologic evaluation permits a more accurate index of viability of fibers than do the biochemical methods utilized.

Cellular death is not so much dependent upon depletion of biochemical reserves as upon the irreversibility of the depletion. This irreversibility is determined by the integrity of the cell structure.

The slow functional recovery of ischemic skeletal muscle is associated with a prolonged relative ischemia due to stasis, which may delay biochemical restitution in the residual normal fibers.

REFERENCES

1. Harman, J. W. A histological study of skeletal muscle in acute ischemia. *Am. J. Path.*, 1947, **23**, 551–565.
2. Harman, J. W. The significance of local vascular phenomena in the production of ischemic necrosis in skeletal muscle. *Am. J. Path.*, 1948, **24**, 625–641.
3. Bollman, J. L., and Flock, E. V. Changes in phosphate of muscle during tourniquet shock. *Am. J. Physiol.*, 1944, **142**, 290–297.

4. Koletsky, S., and Gustafson, G. E. Fluid loss in rats with tourniquet shock. *J. Clin. Investigation*, 1946, **25**, 744–751.
5. LePage, G. A. Biological energy transformations during shock as shown by tissue analyses. *Am. J. Physiol.*, 1946, **146**, 267–281.
6. Buell, M. V., Strauss, M. B., and Andrus, E. C. Metabolic changes involving phosphorus and carbohydrate in the autolyzing gastrocnemius and cardiac muscles of normal, of thyroxinized, and of adrenalectomized animals. *J. Biol. Chem.*, 1932, **98**, 645–669.
7. LePage, G. A., and Umbriet, W. W. Methods for the Analysis of Phosphorylated Intermediates. In: Umbriet, W. W., Burris, R. H., and Stauffer, J. F. Manometric Techniques and Related Methods for the Study of Tissue Metabolism. Burgess Publishing Co., Minneapolis, 1945, pp. 159–174.
8. Good, C. A., Kramer, H., and Somogyi, M. The determination of glycogen. *J. Biol. Chem.*, 1933, **100**, 485–491.
9. Hoffman, W. S. A rapid photoelectric method for the determination of glucose in blood and urine. *J. Biol. Chem.*, 1937, **120**, 51–55.
10. Winnick, T. Microdiffusion methods based on the bisulfite reaction. II. Determination of lactic acid by oxidation with ceric sulfate. *J. Biol. Chem.*, 1942, **142**, 451–459.
11. MacFarlane, M. G., and Spooner, S. J. L. Chemical changes in muscle during and after ischaemia. *Brit. J. Exper. Path.*, 1946, **27**, 339–348.
12. Fishback, D. K., and Fishback, H. R. Studies of experimental muscle degeneration. I. Factors in the production of muscle degeneration. *Am. J. Path.*, 1932, **8**, 193–209.
13. Schoenheimer, R. The Dynamic State of Body Constituents. Harvard University Press, Cambridge, 1942, pp. 63–65.
14. Embden, G., Carstensen, M., and Schumacher, H. Über die Bedeutung der Adenylsäure für die Muskelfunction. 4.Mitteilung: Spaltung und Wiederaufbau der ammoniakbildenden Substanz bei der Muskeltätigkeit. *Ztschr. f. physiol. Chem.*, 1928, **179**, 186–225.
15. Parnas, J. K. Über die Ammoniakbildung im Muskel und ihren Zusammenhang mit Funktion und Zustandsänderung. VI. Mitteilung: Der Zusammenhang der Ammoniakbildung mit der Umwandlung des Adeninnucleotids zu Inosinsäure. *Biochem. Ztschr.*, 1929, **206**, 16–38.
16. Bielschowsky, M., and Green, H. N. Synthesis of adenosine triphosphate by an enzyme system from ischaemic muscle. *Nature, London*, 1945, **156**, 117–118.
17. Fleischmann, W. The metabolism of damaged cells and tissues. *Cold Spring Harbor Symposia on Quantitative Biology*, 1939, **7**, 290–300.

DESCRIPTION OF PLATES

PLATE 112

FIG. 1. Muscle fibers from skeletal muscle ischemic for 6 hours followed by a recovery period of 3 hours are composed of Bowman's anisotropic disks. Nuclei are pyknotic. Hematoxylin and eosin stain. × 500.

FIG. 2. Zenker's hyaline change is manifest in several fibers removed 3 hours after release from an ischemia of 6 hours' duration. The structureless homogeneity of the cytoplasm and pyknosis of nuclei are evident. Hematoxylin and eosin stain. × 500.

1

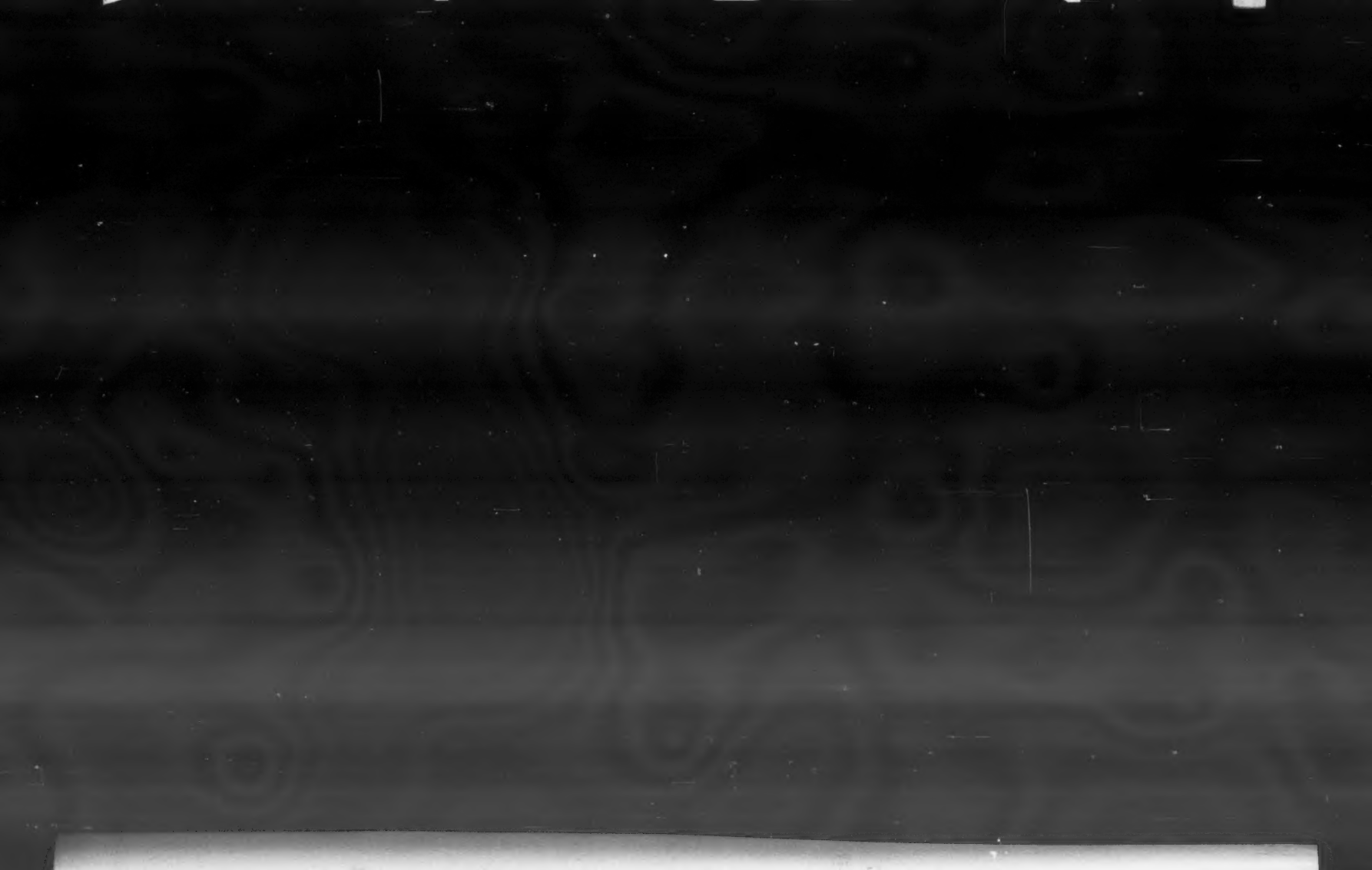
2

Harman and Gwinn Recovery of Muscle from Ischemia

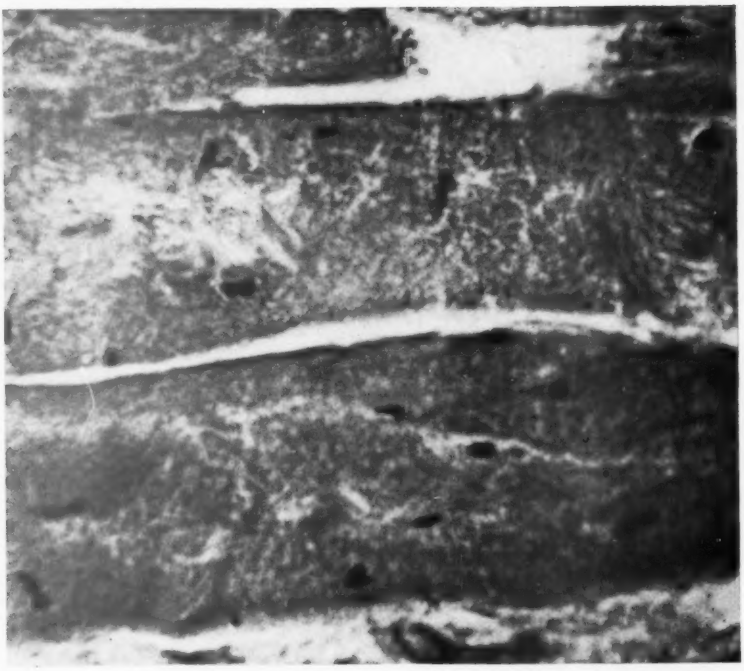
PLATE 113

FIG. 3. The fibers have disintegrated into masses of cytoplasmic granules, a form of granular or "acute molecular" degeneration. The muscle had been released for 3 hours, after 6 hours of ischemia. Hematoxylin and eosin stain. × 500.

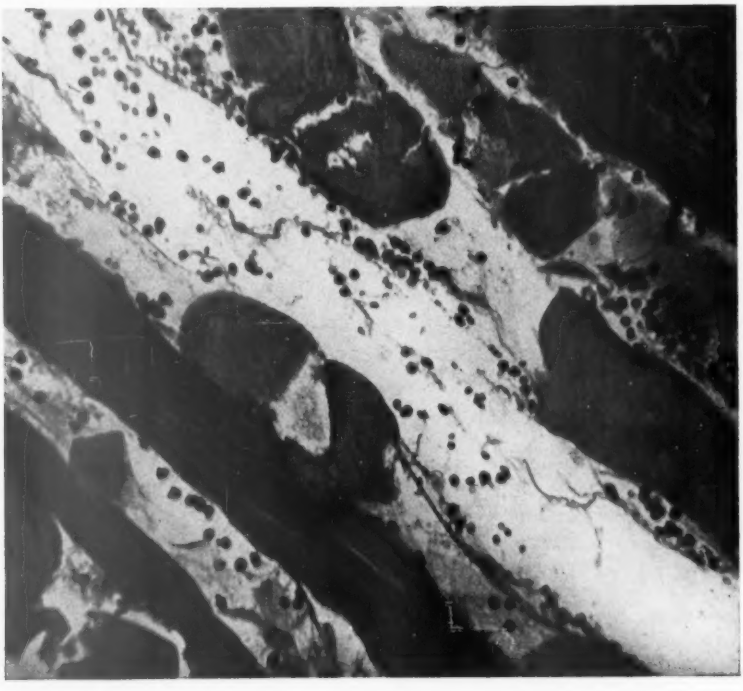
FIG. 4. The tissue is an admixture of normal fibers and others in the process of granular or hyaline necrosis. Both edema and acute inflammatory cells are conspicuous. The ischemia lasted 8 hours and was released for 3 hours. Hematoxylin and eosin stain. × 400.



3



4



BLOOD CYSTS ON THE HEART VALVES OF INFANTS*

T. A. B. Boyd, M.D.

(From the Department of Pathology and Bacteriology, University of Toronto, Toronto, Ont.)

Blood cysts occur frequently on the mitral and tricuspid valve cusps of infants, and rarely on the pulmonary or aortic cusps. They are small, rounded, reddish brown, pin-point to pin-head nodules on the submarginal part of the atrial surface of the cusp (Fig. 1), and usually are sessile but may be pedunculated. In different series in the literature, their reported incidence has ranged from 25 per cent (Luschka,[1] 1857) to almost 100 per cent (Fahr,[2] 1906). They have not been found to be related to other congenital anomalies. The cysts seem to disappear with age, as they are uncommon after 6 months of age. Rare cases have been described in children and adults. Elsässer[3] first described these lesions in 1844, and the subject is well reviewed, with complete references, by Levinson and Learner[4] (1932) and Dow and Harper[5] (1936).

Microscopically, they are masses of red blood cells and a few white blood cells packed in a spherical or ovoid cyst, lying in the connective tissue of the cusp, and lined by a single layer of flat endothelium. When large, they project on the atrial surface, and the connective tissue and endothelium of the cusp are stretched over them. They are usually unilocular, but sometimes have small extensions (A, Figs. 2 and 3), or shallow septa.

Their mode of origin is not definitely known, but they have been variously described as hematomas, angiomas, ectatic or dilated blood vessels, regressive blood vessels, and as being due to blood being pressed into crevices on the ventricular surface of the cusp with subsequent fusion of the mouth. All but the last assume that capillaries or blood vessels exist in the cusps, but Harper[6,7] (1940 and 1945) has shown that blood vessels are present only at the base of the cusp.

Several of these cysts were found on the tricuspid and mitral cusps of an infant who had coarctation of the aorta and died 1 hour after delivery. In a study of serial sections of the posterior mitral cusp, two observations were made which supported the last of the explanations given.

Crevices are common along the ventricular surface of the mitral and aortic valve cusps, and are situated between the attachments of the chordae tendinae. Their formation is believed to be aided by the pressure of the blood when the cusps are being fashioned in utero. One of them, (B), is shown in Figures 2 and 3. In Figure 2, the crevice appears to be

* Received for publication, July 14, 1948.

an empty space, but when followed farther (Fig. 3), it communicates with the ventricle by a narrow mouth. It can readily be imagined how this mouth could undergo closure.

In the blood cysts, evidence that closure had actually occurred is illustrated in Figure 4. Two consecutive sections showed a bar of endothelium joining the ventricular surface to the lining of a small blood cyst.

Summary

Blood cysts are commonly present on the heart valves of infants. The evidence from serial sections of a mitral cusp supports the explanation that they result from blood being pressed into crevices on the ventricular surface of the cusps. Subsequent fusion of the mouths of the crevices forms the blood cyst.

The photographs were taken by Miss J. G. Brown.

References

1. Luschka, H. Die Blutergüsse im Gewebe der Herzklappen. *Virchows Arch. f. path. Anat.*, 1857, **11**, 144–149.
2. Fahr, E. Über die sogenannten Klappenhämatome am Herzen der Neugeborenen. *Virchows Arch. f. path. Anat.*, 1906, **184**, 274–284.
3. Elsässer, C. Bericht über die Ereignisse in der Gebäranstalt des Catherinen-Hospitals im Jahre 1844. *Med. Correspondenzblatt*, 1844, **14**, 297. (Cited by Luschka.[1])
4. Levinson, S. A., and Learner, A. Blood cysts on the heart valves of new-born infants. *Arch. Path.*, 1932, **14**, 810–817.
5. Dow, D. R., and Harper, W. F. Blood cysts in human cardiac valves. *J. Anat.*, 1936–37, **71**, 117–123.
6. Harper, W. F. Further observations on the structure of human heart-valves. *J. Anat.*, 1940, **75**, 88–94.
7. Harper, W. F. The structure of the heart valves, with special reference to their blood supply and the genesis of endocarditis. *J. Path. & Bact.*, 1945, **57**, 229–238.

Description of Plate

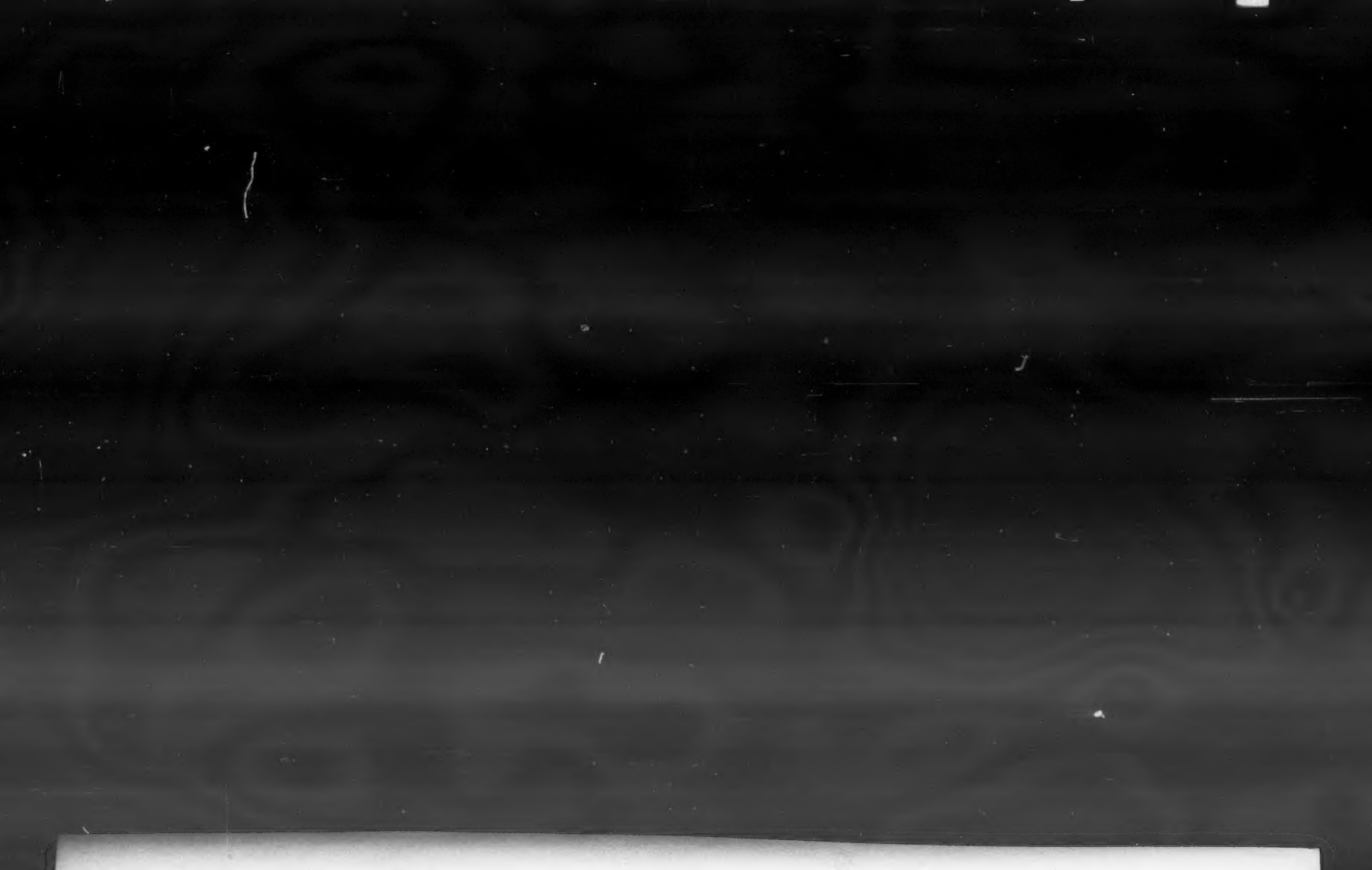
Plate 114

Fig. 1. Blood cysts (A) on the mitral valve. Approximately × 15.

Fig. 2. Space (B) represents the crevice. A large blood cyst projects on the atrial surface and two small ones (A) are embedded in the cusp. Hematoxylin and eosin stain. × 65.

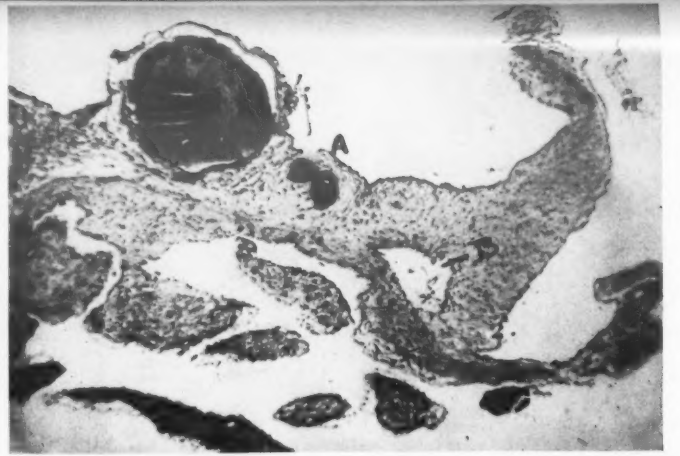
Fig. 3. Crevice (B) joins the ventricle by a narrow mouth. One small blood cyst is seen to arise as a bud from the other. Hematoxylin and eosin stain. × 65.

Fig. 4. A bar of endothelium joins the lining of the small cyst to the ventricular surface of the cusp. Hematoxylin and eosin stain. × 328.

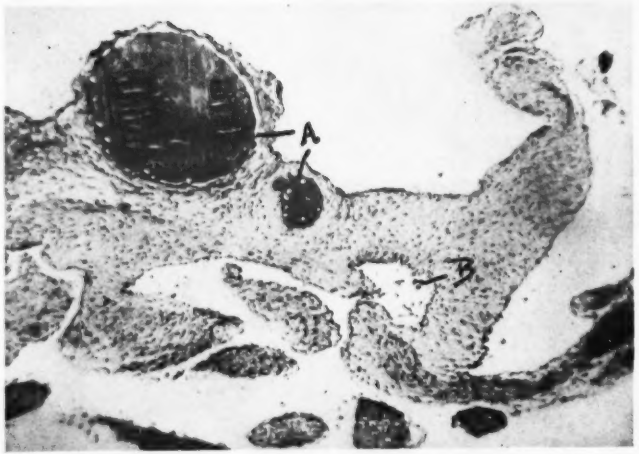


1

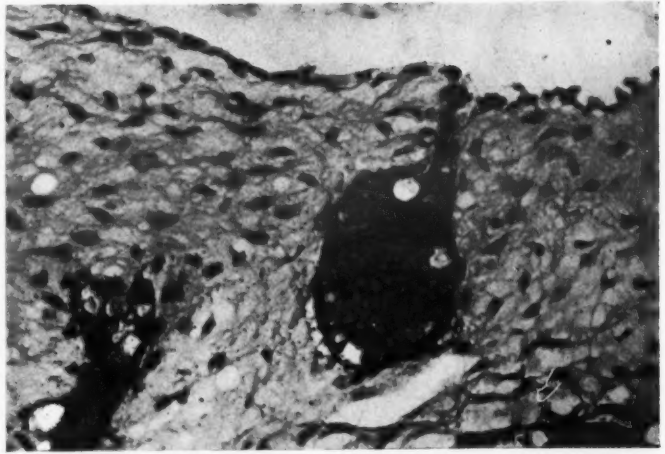
2



3



4



Boyd

Blood Cysts on Heart Valves

FORTY-SIXTH ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

BOSTON

APRIL FIFTEENTH AND SIXTEENTH, 1949

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-Sixth Annual Meeting
Harvard Medical School, Boston, Massachusetts
April Fifteenth and Sixteenth, 1949

PRESIDENT GOODPASTURE IN THE CHAIR

BUSINESS MEETING

April Fifteenth, 1949

Upon nomination of the Council, the Association elected the following officers:

President	SHIELDS WARREN
Vice-President	TRACY B. MALLORY
Secretary	ALAN R. MORITZ
Treasurer	SIDNEY FARBER
Incoming Member of Council	JAMES B. MCNAUGHT

For the council, the President announced the following actions:

Election of new members

A. J. Blanchard, Toronto
George Brecher, Bethesda
John R. Carter, Iowa City
William J. Cromartie, McKinney, Texas
David D. Dahlin, Rochester, Minn.
James R. Dawson, Jr., Nashville
Frank R. Dutra, Cincinnati
Oscar Felsenfeld, Chicago
Russell S. Fisher, Boston
John H. Fodden, Halifax
John P. Fox, New York City
David G. Freiman, Boston
Sidney A. Gladstone, New York City
Abner Golden, Atlanta
John W. Harman, Madison
Maurice Lev, Chicago
Thomas G. Morrione, New York City
Conrad L. Pirani, Chicago
Lelland J. Rather, San Francisco
Arnold J. Rawson, Philadelphia
John D. Reese, McCain, N.C.
Robert J. Ritterhoff, Cincinnati
S. Stanley Schneierson, New York City
William E. Smith, New York City
Sheldon C. Sommers, Boston
Sophie Spitz, New York City
William H. Sternberg, New Orleans
Herbert C. Stoerk, Rahway, N.J.
Lotte Strauss, New York City
Benjamin B. Wells, Houston
C. Merrill Whorton, Atlanta
Irving Zeidman, Philadelphia

Confirmation of a postal vote to reinstate to membership, at his own request, Dr. Charles Oberling.

Acceptance, with regret, of the resignations of Drs. T. S. Cullen, M. S. Fleisher, Russell L. Haden, N. Paul Hudson, Robert Kinnicutt, Mary B. Kirkbride, O. W. H. Mitchell, Phillips Thygeson, and Mary W. Wheeler.

With deep regret, the recording of the deaths of Drs. R. S. Austin, Louis Berger, G. H. Hansmann, George J. Kastlin, Anatole Kolodny, and Robert Koritschoner.

The re-election of Dr. Malcolm H. Soule as Assistant Editor of *The American Journal of Pathology* for the ensuing year, and the re-election of Dr. Shields Warren to the Editorial Board for a period of six years.

The President announced receipt by the Council of a letter from Dr. Karsner in which he expressed the wish to retire from the office of Secretary of the Association. The Council, after serious consideration and with deep regret, had voted to accept Dr. Karsner's resignation from this office. Dr. Sidney Farber read from the floor a resolution of gratitude to Dr. Karsner, and it was voted to spread upon the minutes of this meeting the following resolution, as presented by Dr. Farber:

"The American Association of Pathologists and Bacteriologists has with great regret learned of the desire of Dr. Howard T. Karsner to be relieved of the duties of Secretary because of the assumption of a new position. Out of respect for the expressed wish of one who has done so much for the Association, this wish is accepted. In so doing, the members of the Association make public acknowledgment of the contribution of Dr. Karsner to the very existence of the Association and extend to him our thanks. It is hoped that Dr. Karsner will continue to give the Association the benefit of his wise counsel and advice and that he will remain active in the field of pathology for many years."

The President announced that the next meeting of the Association will be held in Madison. The dates of the meeting will be decided later and will be published in *The American Journal of Pathology* when decision is made. The topic for the symposium for the 1950 meeting is "Reactive Abnormalities of the Vascular and Supporting Tissues with Special Reference to the Collagen Diseases." Dr. Paul Klemperer has been appointed to act as referee.

REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted. It was accompanied by a certification from C. A. Estes, Public Accountant, Boston. In condensed form, the Treasurer's report follows:

General Checking Account

Receipts

Balance on hand, January 1, 1948		$ 2,183.13
Membership dues:		
Current year	$ 7,550.00	
Previous year	70.00	
In advance (1949)	10.00	
Interest on bonds, from investment account	500.00	
		8,130.00
		$10,313.13

Disbursements

American Journal of Pathology ($8.00 per member)	$ 7,120.00	
C. E. Lennon (Secretary to Dr. Karsner)	150.00	
P. A. Glass (Secretary to Dr. Moritz)	150.00	
Reporting 1948 meeting	140.75	
National Academy of Sciences	500.00	
Officers' expenses to meetings	252.20	
Auditing services	35.00	
Bank charges	2.49	
General office expense, secretary, including expense of annual meeting	601.91	
General office expense, treasurer	51.66	
		9,004.01
Balance on hand, December 31, 1948		$ 1,309.12

Investment Account

Balance, January 1, 1948	$34,313.24
Interest on bonds, 1948	500.00
Interest from savings banks	230.07
	$35,043.31
Transfer to checking account (bond interest)	500.00
Balance, December 31, 1948	$34,543.31

Inventory

U.S. bonds 2½, series G	$20,000.00
The Provident Institution for Savings	4,053.46
Franklin Savings Bank	4,060.20
Cambridge Savings Bank	4,311.55
National Shawmut Bank	2,118.10
Total, December 31, 1948	$34,543.31

SCIENTIFIC PROCEEDINGS

INFARCTION OF THE MUSCLE BUNDLES OF THE ATRIA AND VENTRICLES OF THE HEART. William B. Wartman and (by invitation) John Souders, Chicago, Ill.*

CARDIAC HYPERTROPHY IN YOUNG RATS FOLLOWING RENAL LIGATURE: RATE OF DEVELOPMENT AND EFFECT OF SIMULTANEOUS ADRENALECTOMY. Lelland J. Rather (by invitation), San Francisco, Calif.

Abstract. In albino rats subjected to the Grollman technic for inducing hypertension (unilateral nephrectomy and ligature around the remaining kidney), cardiac hypertrophy developed with great rapidity. In a series of animals killed in groups at intervals of 2 to 120 days postoperatively, a statistically significant increase in heart weight appeared as early as 2 days and one-half of the ultimate increase in heart weight was reached in 10 days. No significant progression of hypertrophy occurred after 40 days. Three of 8 surviving rats at 120 days had developed fibrinoid arteritis of the periarteritis nodosa type, involving extrarenal vessels of 200 to 1000 μ in diameter. None of the animals developed glomerular sclerosis or necrotizing arteriolitis of the type seen in malignant hypertension. Control series of sham-operated and unilaterally nephrectomized rats were used. Comparably prepared animals subjected also to bilateral adrenalectomy showed significant cardiac hypertrophy at 10 days but at 20 days the hearts of these animals were only slightly larger than those of control animals. It is suggested that in the adrenalectomized animals the hypertensive load was not maintained.

THE OCCURRENCE OF ARTERIOSCLEROTIC LESIONS IN PYRIDOXINE-DEFICIENT MONKEYS. J. F. Rinehart and (by invitation) L. D. Greenberg, San Francisco, Calif.

Abstract. One year ago we submitted the initial observations on the occurrence of arteriosclerotic lesions in the pyridoxine-deficient rhesus monkeys.† At the present time we have studied 10 animals subjected to pyridoxine deficiency. The basic experimental diet is composed of sucrose, 73 per cent; vitamin test casein, 18 per cent; salt mixture, 4 per cent; and corn oil, 2 per cent. When fully supplemented with known vitamins it has supported excellent growth and the animals have remained vigorous and active. In the pyridoxine-deficient animals degenerative and proliferative lesions with fundamental similarities to those seen in human arteriosclerosis have occurred with great regularity. The severity and extent of the lesions have varied with the duration of the experiments which have ranged from 5½ to 20 months.

In the aorta, lesions are first encountered in the lower abdominal segment. Relatively severe lesions are seen in the iliac and femoral vessels. At the same stage such changes have not occurred in the innominate and carotid arteries. A considerable number of animals have developed significant lesions in the coronary arteries and most of the visceral arteries have been involved. The basic lesion seems to involve a loosening of the intima accompanied by proliferation of connective tissue cells. A mucinous ground substance staining red with thionin lies between the separated fibers. The presumption is that the adhesive quality of the ground substance is lost. As in the human disease, eccentric distribution of the sclerotic plaques and a tendency toward localization at areas of bifurcation are

* Read by title in the absence of the authors. Abstract appears with those of other papers read by title.

† A report of these findings appeared in *The American Journal of Pathology*, 1949, 25, 481–491.

often seen. In a few instances degenerative changes have been seen in the media. In the aorta and iliac vessels, splitting and reduplication of the internal elastic membranes have been observed frequently. The sclerotic lesions are widespread and have been of almost regular occurrence in branches of the renal arteries. Here wavy collagenous fibers in the new-formed thickened intima are coarser than commonly seen in other arteries.

In a considerable series of animals subjected to other deficiencies, lesions of the type described have been absent. Spontaneous arteriosclerosis has not been encountered in the rhesus monkey and this animal has been resistant to the cholesterol lesion. There are a number of considerations other than the direct morphologic evidences presented which suggest that pyridoxine deficiency might be a factor in the pathogenesis of human arteriosclerosis.

Discussion

(Dr. Ernest W. Goodpasture, Nashville, Tenn.) Did you see no particular degenerative change in the elastic tissue of the animals? There was no breaking-up, disintegration, or calcium deposit in the elastic tissue?

(Dr. Rinehart) Yes, the elastic tissue has been interrupted or disintegrated in some of these vessels; the new-formed elastic tissue has often appeared imperfect.

(Dr. William R. Platt, Camden, N.J.) According to Dock the intimal coat of the coronary artery in the male is thicker at birth than in the female. I would like to ask Dr. Rinehart whether he noticed any difference in the degree of coronary arteriosclerosis produced in the pyridoxine-deficient male and female monkeys.

(Dr. Rinehart) Some of the changes I have shown are of a type that are often considered to be those of ageing. However, these changes have not occurred in the control animals or in animals subjected to other deficiencies over a long period of time. Most of our animals have been males.

(Dr. G. Lyman Duff, Montreal, Canada) Have you been able to determine whether there is any lipid deposit in the intima?

(Dr. Rinehart) Fat stains have been done on representative lesions and there is surprisingly little fat seen. Some of the stellate cells contain a few fat granules. These animals are getting a very small amount of fat in the diet; it contains only 2 per cent corn oil.

(Dr. Russell L. Holman, New Orleans, La.) Have you speculated as to the rôle of hyaluronidase? Have you tried vitamin E as a potential preventive of the lesion?

(Dr. Rinehart) The animals have all received vitamin E. I think that is one superior aspect of our experiments; in some experiments of others vitamin E was not included in the diet.

In reply to Dr. Holman's other question, we have speculated as to the rôle of hyaluronidase. There is a mucoid material in the intima and media which is apparently not normally or properly formed. I think this ground substance is probably closely related to hyaluronate.

THE LIPID CONTENT OF THE AORTA IN RELATION TO THE NUTRITIONAL STATE AND OTHER CONDITIONS. Alvin J. Cox, Jr., and (by invitation) Charles D. Miller, San Francisco, Calif.

Abstract. Weights of extractable lipid from 250 aortas were compared with standards for individuals of corresponding ages, and the amount of lipid in each case was expressed in terms of the deviation from the standard. This provided objective quantitative measurements which could be compared without respect to age groups. Pronounced increases in aortic lipid were found in the groups of cases of diabetes and of arterial hypertension. The average aortic lipid value was signifi-

cantly lower in individuals who had little stored fat in areolar tissue than in those who were well nourished at the time of death. This confirms similar observations by Wilens. It has not been possible to relate differences in amount of aortic lipid to specific differences in diet.

Discussion

(Dr. Jesse E. Edwards, Rochester, Minn.) Dr. Cox has shown an increase in the lipid content of the aorta in diabetes. His figures tend to show that arterial hypertension has an influence on the deposit of these substances in the aorta. I wonder whether the figures involving patients with hypertension were broken down as to age, since probably the majority of those with hypertension were in the older age groups. Consequently, there might be an age factor which would tend to bring the patients with hypertension into the group of patients with high lipid content.

(Dr. Esmond R. Long, Philadelphia, Pa.) Dr. Cox's comments on the possible practical significance of the lesions he has described remind me of the fact that sometimes starving peoples find some virtue in their deprivation, to make up for what they have gone through. I recall that after World War I some of the German vital statisticians made the point that, although the German people suffered severely in food intake, there was a considerable reduction in the number of deaths from coronary thrombosis. The explanation was offered that, because there was so much less lipid in the diet, they escaped some evils of the type Dr. Cox has just illustrated. I would like to ask Dr. Cox if he knows if the severe malnutrition that has occurred in some parts of the world in recent years has had any effect on the development of atheromatous disease.

(Dr. Cox) In reply to Dr. Edwards' comment, I should emphasize that all of these cases have been compared with standards for the appropriate age so that we feel that the age factor has been eliminated in this way as far as comparison of the cases is concerned. I do not think it is the factor of age which makes the hypertension cases fall into different groups.

With regard to the influence of nutrition in people subjected to starvation, I can only wonder, as Dr. Long has done, whether it could be one virtue of starvation.

CARDIAC CIRRHOSIS. James S. McCartney, Minneapolis, Minn.

Abstract. Cirrhosis in a person who has had one or more bouts of cardiac decompensation is common, but that the cirrhosis is to be attributed to chronic passive congestion is doubtful. One may find cirrhosis of mild or severe grade which can by no means be correlated with the duration of the clinical evidences of heart failure. If one rejects from consideration those cases with cirrhosis and heart failure plus a history of alcoholism, syphilis, acute catarrhal jaundice or of other possible hepatotoxic agents, the number of instances in which cirrhosis may possibly be attributed to heart failure becomes rather small.

During a 6 years' period the diagnosis of cirrhosis has been made 376 times, or in 2.5 per cent of autopsies. I have examined sections stained for connective tissue from 291 of these cirrhotic livers and have rejected 20 cases because they showed no cirrhosis. In the remaining 271 instances some form of organic heart disease was present in 139, or 50 per cent. From the 271 cases I have selected 64 in which historical or other evidence indicated bouts of heart failure. In 30 instances death was attributed to heart disease, in 16 to cirrhosis, and in the remaining 18 to a variety of causes such as fracture or operation. In 36 instances there was some reason to believe that part of the patients' symptoms might have been due to cirrhosis. The persons with histories of one or more bouts of decompensation of the heart had had such symptoms for periods ranging from 1½ months to 17 years. Two patients had congenital heart disease. One died at the age of 8

years and the other at the age of 28 years. The former had a patchy cirrhosis and chronic passive congestion. The latter had a marked portal cirrhosis, but the signs of passive congestion were not found. In 47 of the 64 I considered the cirrhosis as slight, early, patchy, or subcapsular. Two livers showed hemochromatosis and 2, metastatic carcinoma, and so were rejected. Of the remainder, 4 should be rejected because of chronic alcoholism and 1 because there was a terminal acute yellow atrophy. This leaves, at most, 8 in which chronic passive congestion might conceivably be accused of being the cause of cirrhosis. One had syphilis and so should be excluded. Of the other 7 only one showed cirrhosis of more than moderate degree. Four with a moderate degree of cirrhosis had had periods of decompensation for 17, 5, 2, and 1 years and in 2 the duration of cardiac decompensation is unknown. In none of these livers was there a definite increase of connective tissue in the central portion of the lobule. The only one with severe cirrhosis was a patient with congenital heart disease who died at the age of 28 years.

Conclusions. 1. Cirrhosis of the liver and cardiac decompensation are not infrequently associated. 2. When associated there is little, if any, reason to believe that the cirrhosis is the result of the chronic passive congestion.

Discussion

(Dr. Jesse L. Carr, San Francisco, Calif.) Our figures today are that around 4 per cent of all cirrhotic livers are of this type. I wonder what the actual figures for cirrhosis are in your autopsy series.

(Dr. McCartney) The incidence of cirrhosis in routine autopsies in our series is about 2.5 per cent, which is considerably lower than in many other cities of the country, particularly the coastal cities, where I understand it runs in the neighborhood of 5 per cent. Over the years our figures have averaged 2.5 per cent.

GLOMERULAR ISCHEMIA IN LOWER NEPHRON NEPHROSIS AND IN THE HEPATORENAL SYNDROME. A. James French, Ann Arbor, Mich.

Abstract. The morphologic changes usually described in the renal tubular epithelium in lower nephron nephrosis and in the hepatorenal syndrome cannot be considered sufficient to explain the clinical and laboratory evidences of azotemia. Changes have been found in the glomeruli in cases of oliguria and azotemia of other types which were similar to those designated as glomerulonephrosis in cases of eclampsia by Fahr. These glomerular changes, as associated with eclampsia, had been noted by Löhlein and Leusden and were emphasized by Bell. Trueta has stressed the physiologic findings in renal ischemia.

Twenty cases were selected for detailed study from more than 100 necropsies in which similar glomerular lesions were found to some degree. All cases were eliminated which showed complicating renal diseases, such as glomerulotubular nephritis, arteriosclerotic and arteriolosclerotic nephropathy, diabetic glomerulosclerosis, "wire-loop" lesions in cases of lupus erythematosus, amyloidosis, infarction, and pyelonephritis. The diagnoses in the cases which were utilized included: biliary and atrophic cirrhosis, incompatible blood transfusion reactions, sulfonamide hypersensitivity, acute fibrinopurulent peritonitis, acute yellow atrophy of the liver following arsenical therapy, homologous serum hepatitis, acute hepatitis subsequent to septic abortion, crush syndrome, typhoid fever, carcinoma of the endometrium, myeloid leukemia, and Hodgkin's disease.

The glomerular changes consisted of relative ischemia and thickening of the walls of the capillaries. At least in its early stages, the process appeared to be retrogressive and without increase in connective tissue elements. Even when the thickening of the wall was not sufficient to occlude the capillary lumina, the latter were relatively free from erythrocytes. Proliferation of glomerular epithelium and

increased glomerular cellularity were not prominent findings. The tubular changes in these kidneys were comparable to those described by Bywaters and Beall; Bywaters and Dible; Lucké; Lucké and Mallory; Mallory; Coller, Campbell, and Iob; Darmady; and Ayer. In addition, protein casts, gray with routine hematoxylin and eosin stains, were a common finding in our material and were comparable to the casts in cases of hyperglobulinosis, as noted by Teilum. Tubular lipoidosis was not a conspicuous change.

The retrogressive changes in the walls of the glomerular capillaries were attributed to ischemia. Hyperglobulinosis was a prominent clinical finding in many of the most striking cases in the series. It seemed reasonable to assume that a toxic substance, perhaps a split protein product, might explain the renal changes, including the ischemia, in certain examples of the hepatorenal syndrome and of lower nephron nephrosis.

Discussion

(Dr. Bernard Black-Schaffer, Durham, N.C.) Since Dr. French referred to the work of Trueta, I wonder if he was able to find evidence of any functioning juxtamedullary glomeruli.

(Dr. Lee W. Foster, Chamblee, Ga.) Have you observed this lesion in deeply jaundiced infants showing the morphologic changes of lower nephron nephrosis in the absence of the usual clinical symptomatology?,

(Dr. French) In answer to the first question about functioning juxta-medullary glomeruli, we were interested, of course, in the relationship between the outer cortical glomeruli and those in the juxta-medullary region. The changes reported are diffuse and I do not feel that there was a detectable difference between the glomeruli at the juxta-medullary junction and those that were near the capsular surface. I do not feel that we had confirmation of Trueta's work in that regard.

As far as the infants were concerned, we had examples of lower nephron nephrosis, particularly in infants who received sulfonamides. I purposely eliminated them from this report.

"Lower Nephron Nephrosis" a Misnomer for the "Crush" Kidney. J. F. A. McManus and (by invitation) Guy L. Rutledge, Jr., Birmingham, Ala.

Abstract. The kidneys have been examined by histologic, cytologic, and histochemical methods in a series of fatal cases of trauma. Those dying with the "crush" lesion showed the usual features of relatively normal glomeruli; dilated proximal convoluted tubules with débris in their lumina; pigmented and other casts, especially in the distal convoluted and collecting tubules; tubulo-venous thrombi after 3 days; hyperplasia of the granular cells, with adequate fixation and tissue handling. In addition, it is pointed out that there are no colloid droplets in the epithelium of the proximal convoluted tubules, despite the consistent albuminuria displayed in these cases. The lack of athrocytosis is interpreted to mean a metabolic defect of the proximal convoluted tubules. This abnormality is confirmed by the lack of alkaline phosphatase in the epithelium of the proximal convoluted tubules in groups of nephrons and diffuse decrease in the alkaline phosphatase of all the nephrons. It would seem from these observations, in which the functional and morphologic features of the proximal convoluted tubules are regularly impaired, that lower nephron nephrosis is an inexact, if euphonious, term for a diffuse kidney disease.

Variants of Chronic Granulocytic Leukemia. James Butcher and Edward C. Meek, Jr. (by invitation), and R. Philip Custer, Philadelphia, Pa.

Abstract. The manifold expressions of chronic granulocytic leukemia, both clinical and pathologic, have engendered an unnecessarily complex nomenclature to

denote numerous variants of the disease. Some of the terms do not imply any relationship to leukemia and, indeed, certain of the variants are regarded by some authors as being quite different diseases. In an effort to clarify the situation, some of the more variable features of the disease are considered, as indicated in the following tabulation:

Data

Typical		*Variant*
Adult	Age Incidence	Congenital or childhood
Present	Clinical Manifestations	Absent
Increased	Granular Leukocyte Count	Normal or decreased
Predominantly neutrophilic	Type of Granular Leukocyte	Eosinophilic or basophilic
Predominantly mature	Maturity of Granular Leukocyte	Acute myeloblastic crises
Normal or decreased	Erythrocyte Count	Increased
None to moderate numbers	Circulating Nucleated Red Blood Cells	Great numbers
Normal or decreased	Thrombocyte Count	Increased
None to moderate	Associated Megakaryocytosis	Excessive
Excessive granulopoiesis	Bone Marrow	Fibrous or bony replacement

We would like to use primarily the generic term, chronic granulocytic leukemia, and subordinate the type as indicated: congenital or childhood, subclinical, subleukemic or aleukemic, eosinophilic or basophilic, acute relapsing, erythrocythemic, leukoerythroblastic, thrombocythemic, megakaryocytic, and osteosclerotic or myelofibrotic.

Discussion

(Dr. Harrison Latta, Wellesley, Mass.) I would like to ask whether the cases in which the marrow first showed a leukemia and later sclerosis or fibrosis had any corresponding changes in the other sites of infiltration at autopsy.

(Dr. Joseph E. Pritchard, Montreal, Canada) Dr. Butcher referred to osteosclerosis; I am not clear whether he means osteosclerosis or myelofibrosis.

(Dr. Butcher) In answer to Dr. Latta's question, one case in which an early biopsy was typical of leukemia, later developing osteosclerosis, showed leukemic metaplasia of lymph nodes. The nodes were not sectioned in 2 other cases from our older files; one was not autopsied, and we did not perform the autopsy in the second. In the latter instance we can only presume that nodes were not significantly enlarged. Because our data were incomplete, we listed this case as questionable.

As to Dr. Pritchard's question on osteosclerosis and myelofibrosis, 2 cases showed extensive increase in bony trabeculae; another, merely displacement of marrow by fibrous tissue without associated proliferation of bone. Osteosclerosis, as we interpret it, implies proliferation of bony trabeculae, and it is usually associated with some increase in fibrous tissue in the remaining marrow spaces. Myelofibrosis denotes simply fibrous replacement of marrow.

Pathology of Yellow Fever in 1948 Outbreak of Sylvan Form in Panama. Norman W. Elton and (by invitation) Juan Miguel Herrera, Ancon, Canal Zone.

Abstract. In November-December, 1948, 5 patients in Santo Tomás Hospital, Panama City, died within 5 to 8 days of the onset of acute febrile disease associated with icterus of a low grade. Post-mortem examinations were made in all

cases, but since the microscopic liver lesion in the first (7435) was consistent with the fulminant form of epidemic hepatitis, only gross examination was made on the next two (7441, 7445). A small amount of liver was taken from the fourth (7468), and a liberal amount of tissue from several organs of the fifth (7509). Review of the liver sections on consultation, January 14, 1949, disclosed the typical lesion of yellow fever in the last two (7468 and 7509). This diagnosis was promptly confirmed by the Army Institute of Pathology.

All of these cases occurred in farmers living east of the Panama Canal between Bayanno River and the Zone boundary on the Pacific side of the Continental Divide, and in each instance hunting or agricultural work had caused the person to be in contact with the edge of the forest for an appreciable period of time during the week preceding the onset of his fatal illness. Although all 5 cases were epidemiologically and clinically consistent with yellow fever, discussion will be confined to the 3 on which liver specimens are available.

7435. Age 25. Peñoncito. Hunting trip on horseback into mountain forest. Died November 12th after 5-day illness. Chills, vomiting, diarrhea, abdominal pain. Non-icteric on admission. Became delirious, with convulsions and areflexia. Slight icterus noted at autopsy. Liver coarsely mottled, reddish on yellow. Duodenum, jejunum, and upper ileum hemorrhagic.

7468. Age 24. Rio Utivé. Weeding rice on edge of forest. Died December 5th after 5-day illness. Malaise, chill, headache, high fever, vomiting. Injected conjunctivae. Slight icterus, but had been taking atabrine. Slight icterus noted at autopsy. Liver diffusely mottled with small ruddy spots on pale yellow background.

7509. Age 16. Pedregal. Working in field at edge of forest in Tapia district. Died December 30th after 5-day illness. Chill, fever, malaise, general aches and pains, followed by epistaxis, vomiting, headache, and sore throat. Non-icteric on admission. Injected conjunctivae. Liver tender. Icterus noted at autopsy. Liver homogeneously yellow on cut surface. Punctiform hemorrhages in jejunum and ileum.

The diagnosis of yellow fever in cases 7468 and 7509 is based upon the typical microscopic liver lesion of the entity. Councilman degeneration of polygonal cells in all lobules was diffuse with the exception of the cells ringing the central veins and those at the periphery, which were in both regions almost intact. No central zones were obliterated. Stains with Sudan III demonstrated the characteristic intense fatty degeneration of the entity.

The liver pattern in 7435 was atypical and consistent with that occasionally observed in fulminant epidemic hepatitis. Curiously, necrosis involved only the peripheral zones in most lobules, although in some it was complete. Councilman degeneration was not distinctive, but fatty degeneration and inflammatory cellular infiltration were marked.

Discussion

(Dr. Ralph D. Lillie, Bethesda, Md.) Were mouse protection tests done on the sera of the surviving suspects? If the adrenal was obtained from the one case in which you had more than liver for examination, was there any cell necrosis seen in the adrenal cortical cells? I have seen that in at least one yellow fever case.

(Dr. Virgil H. Cornell, Washington, D.C.) Have you ruled out Weil's disease in these studies, in view of the past history of yellow fever?

(Dr. Elton) The adrenals were not studied microscopically. The whole thing happened so quickly that few, if any, mouse protection tests were done before the outbreak had ended, and it was only in retrospect that it was suspected some of the recovered cases might have been yellow fever. They will be studied epidemiologically and, if they have not been vaccinated in the meanwhile, by protection tests. It will be determined what they were doing and where they were the week before they became sick. All cases in which the pathologic diagnosis was made checked epidemiologically as the sylvan form of yellow fever.

As far as Weil's disease is concerned, we never considered it because everything was so characteristic of yellow fever.

(Dr. Cornell) That's what Noguchi thought.

(Dr. Elton) Yes, but more water has passed over the dam since that time.

EXPERIMENTAL TOTAL MIDZONAL HEPATIC NECROSIS. B. Black-Schaffer and (by invitation) David S. Johnson, Durham, N.C.

Abstract. Rabbits given desiccated thyroid powder (0.73 gm. per diem) until a weight loss of 25 per cent is achieved, react to 0.1 cc. of chloroform per kg., given subcutaneously, with total midzonal necrosis of the entire liver. The central and periportal areas are intact except for loss of glycogen in the periportal zones. Numerous and varied control animals given the same dose of chloroform develop central and focal hepatic necrosis. It appears that under the conditions of the experiment, the midzones of the rabbit liver lobules are metabolically different from the central and periportal zones in a qualitative and/or quantitative sense. It is certain that this unique type of liver necrosis may not be explained solely as a consequence of impaired lobular blood supply (Himsworth).

Discussion

(Dr. Norman W. Elton, Ancon, C.Z.) I would like to ask if studies of the disturbance in the bilirubin excretion mechanism were performed in the course of these experiments, and whether the rabbits developed clinical jaundice; if so, what type it was, and its course, because the jaundice in yellow fever, for example, is late in manifesting itself clinically. There is no doubt it is there serologically or chemically, if bilirubin tests are performed, but a central zone involvement is the commonest etiologic factor in jaundice. I cannot quite place what the rôle of a midzonal necrosis would be in affecting the van den Bergh reaction by the ring test technic.

(Dr. Ralph D. Lillie, Bethesda, Md.) I have seen in DDT poisoning in experimental animals, on occasion, zonal necrosis quite sharply limited to the midzone, I think.

(Dr. Black-Schaffer) In answer to Dr. Elton's question, we have not drawn analogies to human disease since we have not studied the process beyond the point of anatomic observations. None of our animals was permitted to survive more than 24 hours. Whether jaundice would occur after that time we do not know. We have had a batch of animals which we tried to maintain but we found ourselves faced with a very difficult problem; the animals had lost 25 per cent of their body weight, their appetite was severely impaired and the use of vitamin B_1 does not improve it, as far as our experience has gone. I find it difficult, therefore, to answer your question, aside from saying that the conclusions I have drawn are all which I am justified in drawing.

Dr. Lillie's comments are interesting. It is true that there are experiments in which focal midzonal necrosis has been described. Himsworth has attempted to explain this on the basis of intralobular circulation. I think that this is the weakest link of his entire thesis. It is very probable, however, that his thesis may be correct in explaining periportal and central necrosis in many forms of intoxication.

VIRUS HEPATITIS OF CAPTIVE WILD BIRDS. H. L. Ratcliffe and Balduin Lucké, Philadelphia, Pa.

Abstract. During the spring of 1946, a hitherto unrecognized form of hepatitis developed among birds at the Philadelphia Zoological Garden. This disease has continued to the present time and has caused the death of over 150 birds which

were representative of 20 Orders and of approximately 40 Families. Usually birds have died during the acute phases of the disease without showing obvious signs of illness. Less frequently the fatal hepatitis occurred in subacute or chronic form, manifesting itself by reduced activity, loss of appetite, and loss of weight. In the acute form the liver was enlarged, sometimes four-fold. Other viscera exhibited no significant gross changes. In the subacute stage the liver also was usually larger than normal but more or less distorted by the formation of coarse nodules in some areas and shrinkage in other areas. With the chronicity of the disease, distortion of the liver became more pronounced. Subacute and chronic hepatitis was accompanied by edema of the subcutaneous tissues and the muscles, accumulation of fluid in serous cavities, pulmonary edema, and dilatation of the heart.

Microscopic sections of these livers suggests that the basic lesion of avian hepatitis is more or less extensive focal necrosis of liver cells throughout the organ. At times this may be so severe that almost every cell appears necrotic and death occurs before an appreciable reaction develops, but usually necrosis has been limited to many smaller or larger foci, in and about which a few leukocytes and many macrophages accumulate. In such livers macrophages also have been increased throughout the capillary bed of the organ. Intact liver cells contained globules of bile pigment. In examples of the subacute disease, necrotic foci were less conspicuous, but regions were found in which the liver cells had disappeared, leaving collapsed reticulum, macrophages, and bile ducts. In other areas the parenchyma was either intact or had formed regenerating masses of large cells. In chronic hepatitis the livers were composed largely of regenerating nodules which lacked lobular arrangement. These were separated by bands of compressed tissue in which bile ducts and macrophages were numerous. Thus, the morbid anatomy and histopathology of this disease simulates, in many respects, the pattern of epidemic hepatitis in man.

Bacteriologic studies of these livers have been negative, and nutritional factors have been excluded. On experimental grounds avian hepatitis has been attributed, tentatively, to a virus. The source of this disease has not been established, but epidemiologic surveys have revealed that spread within the Zoological Garden may be attributed to direct contact, or to contaminated food, and to the activities of keepers in cleaning cages.

Discussion

(Dr. Ernest W. Goodpasture, Nashville, Tenn.) Did you find any histologic evidence of acute destruction of entire lobules in the liver?

(Dr. Lucké) No, we did not. There was in none of the birds a wiping-out of a third or a half of the liver; the lesions were more scattered.

(Dr. Goodpasture) Is the disease easily transmissible from animal to animal?

(Dr. Lucké) By means of cell-free filtrates we have been able to transmit the disease.

(Dr. Goodpasture) What is the incubation period?

(Dr. Lucké) This apparently depends on dosage and varies with the species.

(Dr. Russell L. Holman, New Orleans, La.) Have any of the attendants had infectious hepatitis, or do you think the birds could be the reservoir for infectious jaundice?

(Dr. Lucké) It is impossible for us to be certain whether any of the attendants have had epidemic hepatitis. There are certainly a number who might have had it. We believe that human hepatitis can be transmitted to birds.

ISOLATION OF BRUCELLA FROM APPARENTLY HEALTHY INDIVIDUALS. Leon V. McVay and Frances Guthrie (by invitation), I. D. Michelson, and Douglas H. Sprunt, Memphis, Tenn.

Abstract. In a paper read before this society in 1942 by W. D. Forbus and his associates, it was reported that brucella organisms had been isolated from several cases of Hodgkin's disease. These findings have been confirmed in our laboratory. It was thought that an explanation of this might be that in an endemic area brucella may localize in chronically damaged tissue. Therefore, chronically infected tissue was cultured for these bacteria.

Two hundred and fifty surgical and autopsy specimens were studied bacteriologically for the presence of brucella organisms. In 4 cases, *Brucella melitensis* was isolated, and in 2 others, *Br. abortus* was found. These specimens were studied microscopically and a vasculitis was observed in 4 of them. Eighty-two prostates were cultured. *Br. abortus* was isolated in 2 instances and *Br. melitensis* in an additional 2 cases. Ninety-three fallopian tubes were cultured. From 2 of these *Br. melitensis* was isolated. Seventy-five autopsy specimens consisting of liver, spleen, lymph nodes and fibrosed areas of lung were similarly studied without success. The cultures of the surgical specimens were made by obtaining from the surgeon, in a sterile container, fallopian tubes or portions of prostate which had been removed through the urethra. Autopsy specimens were acquired as aseptically as possible, and the surfaces were treated with a searing iron. All specimens were immediately macerated, extracted, and the tissue extract incubated in bacto-tryptose broth at 37.5° C. in 10 per cent carbon dioxide for 10 days. After 10 days the broth specimens were inoculated on bacto-tryptose agar plates, and these were similarly incubated for 10 days. Unless positive specimens were obtained sooner, this procedure was repeated twice before discarding the specimens as negative. Identification of the organisms was made by (1) the character of the colony on tryptose agar, (2) the form and staining reactions of the individual organisms, (3) sugar reactions, and (4) by agglutination with specific antiserum.

The specimens yielding brucella on culture were studied microscopically. One prostatic specimen could not be evaluated because of widespread malignant involvement. The remaining 3 showed evidence of a non-specific, chronic inflammatory process of moderate degree. There was vasculitis in 2 of the prostatic specimens and, to a lesser degree, in the fallopian tubes. This involved the small vessels: arterioles, venules, and capillaries. There was thickening of the endothelium with eosinophilic infiltration. Microscopic study of the bacteriologically negative specimens failed to reveal this lesion. Perivascular cuffing by lymphocytes, eosinophils, macrophages, and plasma cells was noted. Hyalinization and vacuolization of the walls of many small vessels was observed.

Five of the patients whose tissue yielded brucella have been carefully studied. All gave a clinical history compatible with brucellosis. All of them had lived on farms and had drunk non-pasteurized milk. In one case there was known exposure to Bang's disease. In 3 cases, 3 intradermal brucella antigens applied simultaneously gave strongly positive reactions. In the fourth case, 2 antigens gave positive reactions, while in the fifth case only 1 antigen showed a positive intradermal test. No specific agglutinins could be demonstrated in the serum of any of the patients. In no case was there a history of a recent episode suggestive of active brucellosis.

Discussion

(Dr. Wiley D. Forbus, Durham, N.C.) The experience of this group of workers is to me not a surprising one. If one makes a large survey, and, of course, this is a considerable series, I think almost any population, certainly any population that

is semi-rural, will yield results of a similar sort. If such a survey is made with repeated cultures, then the chances of increasing the number of positives are, of course, greatly increased. In our own studies of the occurrence of brucella in connection with the Hodgkin's cases we had this experience: We might go for a period of a month or 2 months with repeated cultures and get nothing, and then after about the 30th culture, out would come the organism. So this organism does lie around, if I may be permitted to use that expression, in human tissues, but I am not at all sure that it lies around always in chronically infected tissues. Certainly, our experimental experience would indicate that the presence of no form of irritation of the tissues, infection, or what have you, is required for the localization of the organism. In our studies on the hog, the monkey, and a variety of other animals, we have inoculated brucella in great numbers without producing any form of disease. We have studied these animals at autopsy often well over 300 days, and we have been able to isolate the organism from lymph nodes showing no reaction which might be interpreted as chronic inflammation. I say again that the results of the studies of Dr. McVay and his associates are not surprising, but those which might have been predicted, especially since the study was made on a population which undoubtedly is subject to infection by this organism.

(Dr. W. T. S. Thorp, Bethesda, Md.) Does Dr. McVay consider the intradermal test as evidence of brucellosis?

(Dr. McVay) Not by itself.

(Dr. Thorp) Did you make agglutination tests on individuals who were negative to culture?

(Dr. Leo Lowbeer, Tulsa, Okla.) The interesting phenomenon of a subclinical infection caused by a notoriously pathogenic microorganism is well known from animals like goats and non-pregnant cows, both of which may carry enormous numbers of brucella in their milk-ducts without clinical disease. There are also some reports on subclinical infection in men, particularly those exposed to brucella, like veterinarians or packing house employees. These reports are based on positive blood cultures and agglutination in significant titers in asymptomatic individuals. The existence of a subclinical brucella infection is apparently related to the capacity of brucella to survive as an intracellular organism within mesodermal macrophages and in ectodermal cells of such organs as bone marrow, spleen, lymph nodes, tonsils and gallbladder wall, as shown by Theobald Smith, Goodpasture and Anderson, K. F. Meyer, Castaneda, Boak and Carpenter, Buddingh and Womack. This intracellular parasitism is associated with recurrent clinical disease, if the parasite overcomes the defense mechanism of the host. If, on the other hand, the surviving parasite is held in check by the defense mechanisms, there will be no clinical disease: subclinical infection. The occurrence of clinical disease then depends more on the balance between the rate of growth and virulence of the surviving intracellular parasite, and the humoral and cellular defense mechanisms of the host, than on the fact that the parasite survives. Since nothing is known on agglutination and skin allergy in the cases presented, I should like to ask the authors whether the virulence of the cultured brucella was tested by inoculation of guinea-pigs.

(Dr. McVay) We did not check the virulence on the guinea-pig. We did not check the other patients for intradermal reactions; however, in an attempt to compensate for that we selected 100 random patients and obtained 39 positive reactions, of which 5 were strong. In doing them we found 2 patients with active brucellosis who had fever and chills. Certainly the skin test by itself is not indicative of the diagnosis.

CELLULAR CHANGES IN SYSTEMIC LUPUS ERYTHEMATOSUS. Paul Klemperer and (by invitation) Boris Gueft and Stanley L. Lee, New York, N.Y.

Abstract. "Clumps and packets of hematoxylin-stained bodies" in endocardial vegetations of acute systemic lupus erythematosus were described by Gross. Subsequently Ginzler and Fox reported the finding of similar large "hematoxylin-staining bodies" in necrotic lymph nodes of a case of this disease. Both Gross and Ginzler and Fox recognized the probable origin of these bodies from chromatin material. These observations were confirmed in a large series of autopsied cases. Moreover, similar cellular changes and their evolution were observed in widely disseminated sites, such as serosal membranes, spleen, synovia, mediastinal connective tissue, vagina, skin, and glomeruli, always limited to cells of mesenchymal origin.

Histochemical analysis with Feulgen reaction, methyl green, methyl green-pyronin, ribonuclease, and ultraviolet absorption spectrophotometry reveal that the basic nuclear structural alteration is due to partial depolymerization of desoxyribosenucleic acid. (These studies were carried out with the assistance of Drs. Pollister and Leuchtenberger of Columbia University.) These observations suggest that one factor in the morbid process of acute lupus erythematosus is a disturbance of nucleic acid metabolism manifesting itself in depolymerization of the desoxyribosenucleic acid of the cells of the mesenchyme.

Discussion

(Dr. Robert J. Parsons, Oakland, Calif.) I would like to ask what fixative was used, and if these bodies were seen after all fixatives.

(Dr. Klemperer) The hematoxylin-stained bodies can be seen in tissues fixed in any fixative. For histochemical analysis fixation in Carnoy fluid or acetic acid alcohol is preferable. In the last 3 cases material fixed in this way was available for our studies.

POST-PARTUM ENDOMETRIAL HYPERPLASIA IN DIABETIC PATIENTS TREATED WITH STILBESTROL AND PROLUTON. William A. Meissner and (by invitation) Sheldon C. Sommers, Boston, Mass.

Abstract. Pregnant patients with diabetes mellitus are prone to abort, to have premature deliveries, and to have stillborn fetuses. These complications occur in spite of the fact that insulin requirements are adequately met and the blood sugar is kept under control. The occurrence of such difficulties during pregnancy has been assumed to be due to the deficiency of ovarian hormones which some diabetic females may show. On the basis of this assumption, a large number of pregnant diabetic patients have been carried through pregnancy very successfully with the use of large dosages of stilbestrol and proluton during gestation. In a consecutive series of 50 cases treated with such therapy, post-partum uterine bleeding due clinically to hyperplasia of the endometrium occurred up to 1 year following delivery. The bleeding was often severe, requiring transfusion, and in each instance a dilatation and curettage was performed. The endometrium presented no noteworthy gross features other than its abundance. It was yellow, hemorrhagic, and friable. Microscopically, the endometrium uniformly showed marked cystic and adenomatous proliferation of the glandular epithelium with frequent stratification. The individual epithelial cells showed pleomorphism. There was never conclusive evidence of secretory activity. Mitotic figures were not found in every specimen, but in some there were many. The stroma was unusually active and contained thrombosed sinusoids and eosinophilic leukocytes.

The exact nature and cause of the hyperplasia are unknown, although microscopically it suggests a response to excessive estrogenic stimulation. Subsequent cases

treated with a different brand of stilbestrol (possibly a weaker dose) have failed in most instances to show the same complication. It is possible, but unproved, that the condition is due in part to the diabetic state since the hyperplasia in this series was much more pronounced than that found in a control series of postpartum endometrial hyperplasia in non-diabetic females; moreover, the hyperplasia was more pronounced than that reported in non-diabetic women receiving high dosages of stilbestrol during pregnancy. The hyperplasia was not considered precancerous.

Discussion

(Dr. Joseph E. Pritchard, Montreal, Canada) I should like to ask Dr. Meissner if he noted any vascular changes in the endometrium. I should also like to suggest that this condition might be termed heteroplasia rather than hyperplasia to differentiate it from the more balanced overgrowth of the endometrium; they are all lumped together as hyperplasias.

(Dr. Jesse L. Carr, San Francisco, Calif.) In the cases in which there was infant mortality, did you observe any pathologic changes in the ovaries of the dead infants?

(Dr. Meissner) Dr. Pritchard's suggestion of heteroplasia might be good because this condition is different from the usual hyperplasia, and it does seem to be more heterogeneous than those usually seen.

I am sorry we have no figures on the condition of the ovaries in the dead infants. If a woman had a miscarriage she was not hospitalized in our hospital, so the autopsy on the infant would have been performed in another laboratory.

(Dr. Pritchard) How about the blood vessels?

(Dr. Meissner) They showed no changes other than those usually seen in endometrial hyperplasia. There were no changes which could be attributed specifically to the diabetic state.

SYMPOSIUM ON PATHOLOGY IN FORENSIC MEDICINE

PATHOLOGY IN FORENSIC MEDICINE.* Alan R. Moritz, Boston, Mass.

By definition forensic medicine encompasses all those medical facts and procedures that are required to enforce the law and to administer justice. Since there is no part of any of the special areas between anatomy and zoology that is not occasionally called upon to serve the needs of justice, the potential scientific content of forensic medicine is exceedingly broad. Actually it is not as diffuse as might be inferred from the definition. The principal needs of the law for special medical knowledge fall into two main categories, pathology and psychiatry. This discussion concerns the place of pathology in forensic medicine.

There occur approximately 1.5 million deaths per year in the United States. If the causes of death in those areas from which vital statistics are reasonably reliable are representative of the country at large, it may be estimated that about 300,000 deaths per year result from violence of one kind or another, or occur unexpectedly from obscure causes. The existence in most jurisdictions of laws which stipulate that the cause and manner of such deaths shall be investigated by a public official implies that the acquisition of such information is essential to public welfare. Why is this the case?

In this great pool are all of the homicides, all of the suicides, all of the fatal accidents, and a wide variety of unrecognized rapidly fatal diseases. For the majority there is no reliable history. In many the terminal seizure and death was unwitnessed. Obviously there should be an official agency for obtaining the medi-

* By invitation of the Council.

cal facts necessary to prevent murder going unsuspected, to prevent the prosecution of innocent persons for murder because of misleading circumstantial evidence, and to prevent non-recognition of controllable hazards to public health.

As you know, the office of county coroner is responsible for the investigation of such deaths in most jurisdictions. Because in most communities this office is under the control of a person whose qualifications are political rather than professional and whose resources for and interest in scientific work are insignificant, its medical fact-finding ability is characteristically negligible.

It is probably a fact that in the average American community the only murders that are recognized as such are those that were witnessed or those in which the victim shows unmistakable external evidence of assault. It would be interesting to know what the actual homicide rate in the United States may be.

It is also a fact that in the average American community the presence of pathologic changes that simulate violence in or on the body of a person whose death occurred in suspicious circumstances is likely to result in the prosecution of whomever may have had motive and opportunity to kill. I have known of many prosecutions for murder in which the medical evidence pertaining to the cause and manner of death was totally unreliable.

It is, furthermore, a fact that in the average American community there is no mechanism for compelling the performance of a competent autopsy in the interests of public health. In a community such as Massachusetts, in which the interests of public health are sufficient justification for the performance of a compulsory autopsy, many otherwise unsuspected or unproved hazards to life are disclosed by medicolegal autopsies.

What is the responsibility of American pathology in relation to the investigation of these 300,000 so-called medicolegal deaths? At the present moment there are relatively few communities in the United States in which qualified pathologists are utilized for this important public service.

There should be in every community an official organization which would provide, first, for the screening of all medicolegal deaths, and second, for the acquisition of complete and reliable evidence in those instances in which such information is needed in the public interest. To provide a screening mechanism there should be in each community one or more official physicians to whom all deaths by violence or deaths due to obscure causes are reported for preliminary investigation. If the information derived from a preliminary inquiry and view of the body of the dead person indicates that there is no reason to believe that public welfare is jeopardized, the death may be certified without further investigation.

There should also exist in every city and state a facility for conducting complete and competent official post-mortem investigations whenever the need for such information is disclosed by the preliminary inquiry. Experience in Massachusetts where such a mechanism exists indicates that autopsy is required in the interest of public safety in approximately one of every five of the so-called medicolegal deaths.

Unfortunately, too many pathologists are unwilling or unable to assume responsibility for certain objectives of a medicolegal autopsy that are peculiar to the fact that it is performed for medicolegal purposes. Too often the pathologist confines his investigation to what he construes to be the normal scientific objectives of an autopsy and ignores certain evidence that is fully as important from a medicolegal standpoint as any of the conventional medical facts disclosed.

What are some of the special characteristics of forensic pathology?

One deals with problems in identification. In approximately 5 per cent of the medicolegal autopsies conducted by the Department of Legal Medicine of Har-

vard Medical School the first question to be answered relates to the identity of the dead person. This is particularly true in the case of burned, mutilated, or decomposed bodies. Sometimes the problem is even more basic and concerns determination of whether the fragmentary remains are from man or animal? The pathologist who accepts the responsibility for medicolegal autopsies must be prepared either to make such determinations himself or to see to it that the material in question reaches the hands of a person qualified to deal with it.

The aggregate of many little things, no one of which is necessarily significant by itself, is often of great importance in the establishment of identification. The fragmentary remains of a skeleton were recently found partially buried in the humus of a pine forest. Examination of the bones by an anatomist and a roentgenologist established that the remains were those of a young women having a stature of approximately 52 inches. Animals and flood water presumably were responsible for the fact that many of the bones were missing. The only vestiges of the head were one incisor tooth and a mat of brown hair. A dental expert who examined the tooth recognized a highly distinctive dental deformity. The sex, age, stature, and hair were consistent with those of several missing girls but the recognition of the peculiar dental deformity known to have been possessed by one of them established the individual identity of the remains with reasonable certainty. The finding beside the bones of a stoppered bottle which contained traces of barbiturate together with information from relatives that the missing girl had been exceedingly despondent justified the presumption of suicide.

In approximately 5 per cent of medicolegal autopsies the establishment of the probable time of death is of critical importance. The accuracy desired may be in terms of hours, days, weeks, or years according to the circumstances of the case. If John Doe, whose fatal injuries appear to be compatible with suicide, died yesterday, the presumption of suicide may stand. If, however, the medical evidence indicates that death occurred 2 days ago, the fact that neighbors heard someone moving around in the dead man's room yesterday may justify a presumption of homicide. Thus, a knowledge of the rate and sequence of the changes that occur in the human body after death is an important part of the armamentarium of the forensic pathologist. Most of all he should be aware of the limitations of such evidence pertaining to the time of death. In the case of the skeleton found in the woods, the police investigation was greatly facilitated by information supplied by the pathologist in respect to the time of death. It was reported that the skeleton had lain on the ground where it was found for at least 7 years, and probably longer. This information was provided with the aid of a botanist who examined the roots of trees that had grown through the fragmentary remains of the victim's clothing. Birch roots 5 years old were identified and since several years are required for pine needles to decompose sufficiently to support the growth of a deciduous tree, 7 years was estimated as the minimal duration of the post-mortem interval. Actually the body had lain in the forest for 12 years.

Obviously, the determination of the cause of death with the greatest possible accuracy is desirable in all medicolegal autopsies. Too often a medicolegal autopsy is terminated with the finding of what seems to be an adequate explanation of death and no real effort is made to exclude other possibilities. Many elderly persons have degenerative diseases which, in the absence of evidence to the contrary, might be regarded as probable causes of death. A good illustration of the danger of forming a premature conclusion is provided by the case of an elderly woman who was found dead in bed. An autopsy disclosed that she had pulmonary tuberculosis, a cancer of the cervix, and chronic pylonephritis. Further investigation established that her death was actually due to arsenic poisoning. Arsenic

was looked for not because there was anything in the history to indicate such a probability but because of unusual hyperemia of the gastric mucosa and the finding of a small amount of white particulate matter in the gastric contents.

Another occasional cause of a pathologist's failure in the investigation of a medicolegal death is to assume that death was caused by trauma because of the presence of external injuries on the body. Recently a woman with a badly bruised face was found dead beside a car in the parking lot of a country tavern. She and her male companion had spent the evening in the tavern and were drunk and quarrelsome. About eleven o'clock the woman left and 30 minutes later he, too, left. Sometime thereafter another guest going to his car saw the woman on the ground and her companion bending over her. Together they brought her into the tavern where a doctor was called who pronounced her dead. She had a bruised face and scalp and autopsy disclosed an intracranial hemorrhage which was at first considered to be traumatic. The suspect said that he had found the woman on the ground when he went outside to look for her. His story was not believed at first. If death had been reported as due to head injury the circumstances were such as to have led to suspicion, if not prosecution, of the dead women's companion for fatal assault. Actually, a complete autopsy disclosed that death resulted from the rupture of an exceedingly small congenital aneurysm of the circle of Willis.

In the performance of autopsies for medical rather than medicolegal purposes the pathologist is rarely concerned with the acquisition of evidence bearing on the manner or circumstances in which injuries were received. In a medicolegal investigation such evidence may be of paramount importance. Thus the finding of a blood-alcohol level of 0.30 per cent in the pedestrian victim of an unwitnessed fatal automobile accident may confirm the driver's statement that the dead man stepped off the curb directly in front of the car. A pathologist should always be alert to the possibility of illuminating the circumstances that attended the receipt of fatal injuries.

A few months ago a shooting occurred in which the two men involved were approximately 6 feet apart at the time the fatal shot was fired. A 45 caliber bullet passed through the victim's chest from front to back and was immediately fatal. The other man admitted the shooting but stated that it was meant to be a joke and that the gun was not pointed at the dead man when he pulled the trigger. His story was confirmed by the fact that the appearance of the entrance wound in the front of the dead man's chest indicated that the bullet was already badly deformed and exceedingly hot when it first struck him. The finding that the bullet was already both hot and deformed indicated that it had struck some resistant object before striking the dead man's chest. Actually it had first struck a steel shelf and had ricocheted in such a direction as to cause the fatal injury. If this had not been recognized, the death would have led to criminal prosecution.

There are a variety of reasons why pathology participates ineffectively in the practice of forensic medicine in America. One of the most important of these is that in most places forensic pathology is not included in the training of pathologists. A pathologist is not likely to be competent to meet the problems of forensic medicine unless he has had training or experience in dealing with them. We are not going to acquire experience or be able to give adequate training unless we acquire local responsibility for the practice of forensic pathology. If the majority of members of this Association persist in their present relatively passive attitude toward pathology in forensic medicine, the situation will remain unimproved. Those of us who feel keenly the need for improvement are enormously encouraged by the interest evidenced by the allocation of this symposium to a consideration of pathology in forensic medicine.

A Study of the Electric Resistance Offered by Animal and Human Tissue. R. S. Fisher (by invitation) and Alan R. Moritz, Boston, Mass.

Abstract. Studies have been conducted using direct current and silver-silver chloride non-polarizing electrodes with actual skin contact through saline media in glass tubes. The following conclusions are reached:

1. In dealing with tissues, resistance may be expressed in terms of ohms, only for specified electrode areas and lengths of pathway.

2. The skin offers relatively high resistance (700 to 15,000 ohms/3 cm. diameter circular electrode with double thickness skin) to low voltages (6 to 10 volts), but at higher voltages (above 50 to 70 volts) skin resistance becomes very low.

3. Contrary to published reports, internal tissues, especially fat, offer significantly large resistance to the passage of direct current.

4. With intact extremities in humans and trans-thigh sites in pigs, the effective resistance increases as the area of the electrodes is decreased, but the increase is disproportionate to (is less than) the decrease in area. This is explainable by an internal (ellipsoidal) path of larger diameter than the electrode area.

5. Using sodium chloride solutions in the electrodes, there is an apparent asymmetric localization of resistance with that under the cathode being larger than that under the anode. With potassium chloride solutions the anode appears to be the site of higher resistance.

6. Thermal effects (heating) observed under the electrodes appear, in preliminary experiments, to be of sufficient magnitude to explain the injuries incident to the passage of the current.

The Medicolegal Aspects of Radiation Injury. Shields Warren, Boston, Mass.

Abstract. The medicolegal aspects of radiation injury involve the following factors, among others:

1. The pathology of radiation injury.
2. Means of prevention.
3. Responsibility for injuries produced.

As evidence of local radiation injury the triad of (a) wide variation in cellular alterations; (b) increase in, and hyalinization of, connective tissue; and (c) thickening of blood vessel walls or occlusion of blood vessels with or without telangiectasia, is helpful. With atrophic changes in hematopoietic tissue or osteogenic sarcoma in an adult, radiation injury, either external or internal, should be considered. It is the responsibility of each individual handling apparatus or material giving off ionizing radiation to see that he himself, his staff, his patients, and those working nearby are adequately protected.

Blunt Force Injuries of the Heart. Milton Helpern, New York, N.Y.

Abstract. Traumatic injuries of the heart from non-penetrating blunt force result only when a severe grade of violence is applied directly to the chest wall. The force may be generalized or localized. In most cases cardiac injuries are associated with other severe chest and bodily injuries and are immediately or rapidly fatal. Generalized blunt force, severe enough to crush or compress the chest and produce cardiac and other intrathoracic injury, is encountered most often in vehicle accidents and in falls from a height. A crushing downward force applied anteriorly may tear the heart more or less completely from its superior and posterior attachments and also tear the pericardial sac. In young persons with a resilient thorax, this may occur without fracture of the ribs or sternum. With less violence, incomplete internal transverse lacerations of one or both auricles above the auriculoventricular valve rings, and of the attachments of the venae cavae and pul-

monic veins may occur; if the victim survives long enough, mural thrombi may develop over the site of injury in the auricle.

Crushing of the chest may produce punctures and lacerations of the pericardium and heart by sharp ends of broken ribs or sternum. There may be a variety of cardiac injuries including tears of the valve cusps and leaflets and of the papillary muscles. Contusions of the auricles or ventricles and injuries of the coronary arteries are rarely encountered and never as isolated injuries. Incomplete internal laceration of the right coronary artery just beyond the ostium was one of multiple severe cardiac injuries in 2 cases. Injuries of the left coronary artery have not been encountered. The anterior position of the right coronary artery makes it more vulnerable than the left. Traumatic injury of a calcified coronary artery and traumatic coronary thrombosis have not been encountered in a large series of blunt force cardiac injuries observed at autopsy.

Sudden violent compression of the chest may produce one or more bursting ruptures of the heart, involving the ventricles, auricles, or septa. The pericardium may not be torn and in young persons the bony thorax may escape fracture. Death ensues rapidly from intrapericardial hemorrhage. Bursting ruptures are the most frequent cardiac injury resulting from falls from a height. Rarely, severe compression of the chest may cause a long bursting laceration of the pericardium and relatively slight contusion of the right auricle or ventricle; in such cases the victim, even with a markedly hypertrophied heart and severe coronary sclerosis, may survive many days and death result only in part from the cardiac injuries.

A severe localized blunt force like that from the impact of a small, hard, fast-moving object, striking the chest over the sternum or just lateral to it on either side, may contuse and rupture the thin anterior wall of the right ventricle without damaging the pericardial sac. The right ventricle is especially vulnerable to violence of this type because of its anterior position. The force is usually severe enough to fracture the costal cartilages or the sternum beneath the site of impact. The ruptures are usually small and, if complete, result in rapid death from hemopericardium. In some cases, rupture of the contused area is delayed several hours or days. In rare cases a contusion may heal with or without formation of a small aneurysm and mural thrombus. The great majority of isolated contusions of the heart occur in the anterior wall of the right ventricle as the result of such localized blunt force impacts.

Traumatic injury of the heart by the mechanism of contrecoup with the force transmitted obliquely upward from the buttocks does not occur and does not merit serious consideration. Bursting ruptures of the heart do not result from indirect violence which compresses the abdomen.

Discussion

(Dr. William R. Platt, Camden, N.J.) I should like to ask Dr. Helpern how he feels about the relationship of trauma to the sternum in young people and the subsequent development of anginal attacks leading to coronary disease and death in a 6-months' period.

(Dr. John J. Clemmer, Albany, N.Y.) Has Dr. Helpern seen pericarditis following injuries of the chest?

(Dr. Victor Levine, Chicago, Ill.) I should like to ask if a less severe injury to the chest might cause damage and laceration of the coronary artery if the artery is very sclerotic.

(Dr. Helpern) The answer to the first question is difficult in the absence of the post-mortem evidence. We have found severe coronary sclerosis not infrequently as a cause of sudden natural death in young adults in whom the disease was entirely asymptomatic and therefore unrecognized and unsuspected during life. It is true that blunt force injury to the chest might precipitate symp-

toms in a person with pre-existing, but previously asymptomatic, coronary disease but it does not cause the disease. It is a mistake to assume that because there have been no prior symptoms there has been no pre-existing disease. Most of the cases we have seen in which symptoms developed and progressed fatally after injury revealed evidence of severe pre-existing coronary arteriosclerosis.

With regard to pericarditis following an injury to the chest, I do not recall any case in which pericarditis developed as a complication of a non-penetrating blunt force injury, although it is conceivable that it might develop as an indirect complication of infection extending from the lung or pleural cavity, or as a manifestation of sepsis complicating infection elsewhere in the body.

In reply to the third question as to a less severe injury to the chest causing damage and laceration of a very sclerotic coronary artery, I should like to emphasize that the coronary arteries whether normal or sclerotic are rarely injured even by the most severe blunt force; therefore lesser degrees of force would also have no direct effect. In the 2 cases which I demonstrated the coronary artery injury was only one of many other cardiac and thoracic injuries. It was not an isolated lesion. I have never seen the left coronary artery similarly injured.

SUBDURAL HEMORRHAGES FOLLOWING RUPTURES OF ARTERIES AND VEINS ON SURFACE OF CEREBRUM. Benjamin Morgan Vance, New York, N.Y.

Abstract. The findings were reviewed in deaths from subdural hemorrhage due to a rupture of a blood vessel on the surface of the cerebral hemisphere, as encountered among the autopsies in the Office of the Chief Medical Examiner in New York City. Such deaths are important forensically, inasmuch as some of the subjects have been exposed to criminal or accidental violence, and, in cases of this type, it is essential that the lesions causing death should be accurately determined and their connection with trauma properly appraised. Microscopic examinations were made in 10 cases and in 6 the ruptured blood vessels were demonstrated satisfactorily, 3 being arteries and 3 veins. The mechanism of the rupture of blood vessels is discussed.

Discussion

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder whether Dr. Vance noticed herniation of the uncinate gyri in these cases of subdural hemorrhage. The reason I ask that question is that therein may be the mechanism for the pontine hemorrhages that are found in association with them. I think such pontine hemorrhages can be produced experimentally by subdural injection of fluid foreign bodies of different sorts. In the group of cases where arterial rupture occurred, and I realize there are very few, is it Dr. Vance's impression that the clinical picture is a more rapid one in contrast to the slower venous bleeding into the subdural space?

(Dr. Vance) To answer Dr. Angrist's last question first, it seemed to me that fatal subdural hemorrhages from ruptured arteries developed in about the same length of time as subdural hemorrhages from ruptured veins. However, as I have investigated only 6 cases in which the ruptured blood vessels were identified as arteries or veins, I cannot speak with authority on this point. As for the uncinate gyri, I do not recall having seen them unduly prominent in cases in which discrete pontile hemorrhages were present.

THYMIC HYPERTROPHY AND AURICULAR COMPRESSION. Jesse L. Catt, San Francisco, Calif.

Abstract. In a post-mortem study of approximately 1500 autopsies on babies under 1 year old, dying suddenly, and worked up at the San Francisco Coroner's Office, numerous causes of death have been found, and among them there have been included lymphatism, status thymico-lymphaticus, status thymico-asthmati-

cus, and combinations of these three. In a certain percentage of children dying with a large thymus and with the mechanism of death otherwise unexplained it has been reasonably possible to ascribe death to compression by a large thymus of the auricles, particularly on the right side. In a relative sense, the auricles in these children are appreciably smaller than in normal babies of the same age, and at autopsy the auricles are found to be empty. There are also certain other upsets in the dynamics of circulation in these children, which we believe to be of importance in explaining their sudden demise. This material, together with microscopic studies of the lungs, heart, and lymphatic system, is included.

Discussion

(Dr. Jesse E. Edwards, Rochester, Minn.) I should like to point out that in certain newborn infants with serious respiratory difficulties, dissection of the thymus, even a large thymus, from the mediastinal structures will uncover a malformation of the aortic arch which compresses the trachea and which is the basis for the respiratory distress. That this point is stressed is justified by the fact that for some of the vascular malformations to which I refer there are available surgical technics which may be lifesaving, if employed.

(Dr. Jacob Werne, New York, N.Y.) In 1942 is was shown before this Society that a series of normal infants killed instantly by violence showed as large, or larger, thymic glands than any which have been described in so-called status thymico-lymphaticus. If Dr. Carr will autopsy infants dying of violence in his jurisdiction, he will confirm the fact that a normal infant's thymus is large. With respect to the thymic hemorrhages, I will have more to say during our presentation of histopathologic study of actual and alleged asphyxial deaths. The thymic petechiae seen in so-called status thymico-lymphaticus are referable to vascular injury resulting from fulminating infection, usually of respiratory origin.

(Dr. Frank Dutra, Cincinnati, Ohio) One fact which leaves me totally unconvinced on this matter is that these children die suddenly without any evidences of cardiac decompensation. In the ordinary situation where there is a tumor growing against and compressing the right auricle there is the development of marked peripheral evidences of chronic venous stasis. There is no evidence in any of the cases presented that there has been any such stasis, in spite of this alleged severe pressure on the right auricle.

Anyone who does autopsies knows that it is common to see hemorrhages scattered throughout the thymus as a terminal event, and this is absolutely insignificant as a factor in predisposing death. Small hemorrhages are commonly found in the thymus when death was preceded by anoxemia or infectious disease.

I should like to say one thing more. In order to demonstrate abnormal anatomic relationships, the organs must be examined before those relationships are destroyed. Certain conclusions about the possibility of one organ compressing an adjacent one would be totally invalid if the observations on which they were based were not made while the organs were *in situ*. Even removal of the viscera *en bloc* would distort relationships, and organs which had been dissected elsewhere and sent in for examination could not possibly yield information which would be useful in a study of the kind reported here.

I am concerned with the re-implication of abnormality of the thymus as a cause of sudden death, because when it was accepted it was used only as a mask for the ignorance of those pathologists who were inept at making accurate examinations, or to protect the pride of those who were unwilling to admit, after the autopsy, that they did not know the cause of a death.

(Dr. Carr) It is a pleasure to have started the first fight of today. I am complimented that I leave at least two people cold. No one, in fact, seems to be very hot about the subject. I should like to answer these questions in sequence, and in

answer to the first question, may I say that we agree with Dr. Werne's statement about anomalies, but wish to note that congenital anomalies of the circulatory system, particular of the coronary vessels and great vessels of the neck, are far more common with the thymic status than in normal babies. His second suggestion that normal infants have large thymuses is also true, and it is our experience that the thymic size in babies is actually far larger than the published figures. The thymic weight also varies with the baby's nutrition, with its heritage, and with geographic distribution, but the doctor missed the point entirely, because size actually has nothing to do with auricular compression, nor does size alone have to do with death in status thymico-lymphaticus. Large thymuses may be large and compatible with normal function. They may also be large and associated with other related changes, which cause death. As far as the figures he cites are concerned, and his suggestion that we should do autopsies on normal babies to find out the thymic size, may I state that we do many autopsies on normal babies and find large thymuses. We also do autopsies on babies who have died by accident, who have large thymuses. But, may I call your attention to the fact that these babies that I have reported had large thymuses and no accidents, and that they are still dead. I would suggest that he do more autopsies on babies which do not die by accident, to find the normal size under other circumstances. We cannot agree at all with the statement that thymic hemorrhages are commonly found in routine autopsies in babies dying of accident or disease. We would be very happy to match figures with Dr. Werne, because we have, in autopsies on 1500 babies, 135 cases of this sort. As far as Dr. Dutra's criticism of the selection of cases is concerned, we are quite conscious of the quality of our material, and agree that material sent in from the outside is the worst kind of material we can get, but we chose it specifically for this initial presentation and reopening of this subject, because it left us personally out of the picture, and as I said before, eliminated the geographic imputation to some degree.

HISTOPATHOLOGIC DISTINCTIONS BETWEEN ACTUAL AND ALLEGED ASPHYXIAL DEATH DURING INFANCY. Jacob Werne and (by invitation) Irene Garrow, New York, N.Y.

Abstract. In a previous investigation of 167 consecutive infants under 1 year of age found dead in crib, carriage, or bed while in apparent health, the authors found, as the most common cause of death, fulminating respiratory disease. The sudden death in these cases is not due to accidental mechanical suffocation but represents a further development of the circulatory collapse that not infrequently characterizes severe infections. Since such cases are ordinarily still certified as due to accidental mechanical suffocation, a comparison of the lesions found in this group with those encountered in infants dying of known asphyxial causes is reported. Fifteen consecutive cases, 1 month to 4 years of age, found dead of drowning or strangulation, failed to show significant tracheobronchitis, otitis media or mastoiditis, or interstitial pneumonitis, and showed relatively slight congestion, edema, and hemorrhages of the lungs as compared with the group allegedly suffocated by bedclothes. Also conspicuously absent in the known asphyxial deaths were the extreme visceral congestion and foci of cellular infiltration and parenchymatous degeneration which we have encountered in infants dying of fulminating respiratory disease.

AIR EMBOLISM AS A COMPLICATION OF LUMBAR PNEUMO-ENCEPHALOGRAPHY. REPORT OF TWO CASES WITH NECROPSY FINDINGS. Frank C. Coleman, John R. Schenken, and Walter D. Abbott, Des Moines, Iowa, and Omaha, Nebr.

Abstract. Two cases of fatal air embolism are reported as a complication of lumbar pneumo-encephalography. Symptoms began in each case near the com-

pletion of the procedure, and death followed within a few minutes. Two factors seem to be responsible for the condition: (1) canalization of an epidural vein, and (2) the pressure of the injected air must be greater than the venous pressure.

Discussion

(Dr. Walter Putschar, Charleston, W. Va.) We have seen a case similar to those presented during an encephalographic procedure. The child was in bed, air was being injected, and the child died with a stoppage of the heart under continued respiration. In this case the spinal cord was removed and we could not demonstrate any openings in the veins. However, we assumed that in some way the needle must have gotten into the venous plexus. The heart was distended with air, and there was no doubt that it was an example of fatal air embolism.

SOME SIGNS, FINDINGS, AND INTERPRETATIONS OF CRIMINAL ABORTION. William E. B. Hall, St. Louis, Mo.

Abstract. 1. Massive hemolysis following hemolytic abortefacients. Three cases were studied in which ingestion of abortefacient liquids was followed by massive hemolysis with the production of hemoglobinuria and red-stained saliva, tears and perspiration, the latter copiously staining clothing, sheeting and pillows, ultimately followed by death in approximately 2 days. A listing is made of liquid abortefacients capable of causing hemolysis by ingestion, absorption, or parenteral injection and sometimes by intra-uterine injection.

2. Bacteremia in the embryo and placenta. Bacteria demonstrated in ample quantities in the vessels of early embryos are interpreted as indicating traumatic damage to the chorio-amniotic sac with entry into the fetal vascular system prior to death. As such their presence is a presumptive sign of an infected abortion produced by pre-abortional interference.

3. Postabortional uterine myiasis. A case of abortion is reported in which autopsy showed the uterine fundic cavity to be occupied by a number of second stage larvae of the blue bottle fly. Their discovery was in corroboration of the production of abortion by the instrument used by the patient—a stick obtained from the barnyard manure pile. Further, the identity of the larvae and their stages of development served as excellent indicators of the approximate time of abortion.

Discussion

(Dr. Frederic Wohlwill, Hathorne, Mass.) Regarding the last item, I would like to emphasize that in these cases we deal with a fetal septicemia with infected emboli in the capillaries of the chorionic villi, leading to an "intravillous placentitis." As has been mentioned, the fetus will, in the majority of cases, be expelled before the mother has been infected. But when we studied this affection in 1928 we found that in 2 cases in which *Eberthella coli* was the agent the mother had been secondarily infected. In one of these she died of *E. coli* septicemia.

PATHOLOGY OF ACUTE CARBON TETRACHLORIDE TOXICITY. Henry D. Moon, San Francisco, Calif.

Abstract. This study was based on 12 fatal cases of acute carbon tetrachloride poisoning resulting from inhalation or ingestion. In 11 of the 12 cases there was associated acute or chronic alcoholism. The duration between poisoning and death varied from 4 to 18 days, thus affording an opportunity to study the morphologic changes in the liver and kidney at various intervals following poisoning. The hepatic lesions were characterized by early severe central necrosis of the lobules followed by an inflammatory reaction and regeneration which occurred almost simultaneously. The renal lesions in patients dying within a few days of poisoning were relatively slight and the pathologic changes were those of moderate cloudy

swelling. In the later stages tubular degeneration was profound and resembled the picture seen in the kidney of the "crush syndrome." Pulmonary edema occurred clinically as a late manifestation and was present in nearly all cases at autopsy. A diagnostic triad of central lobular necrosis of the liver, renal tubular degeneration, and pulmonary edema was consistently present in this series of patients.

Discussion

(Dr. Alfred Angrist, Jamaica, N.Y.) I should like to ask about the relationship of alcoholism to carbon tetrachloride poisoning. In the 2 instances I have seen, chronic alcoholics who did not stop taking alcohol succumbed more rapidly to exposure to carbon tetrachloride than did normal individuals without a history of alcoholism. I understand from your chart, Dr. Moon, that you found several such cases. The point is of considerable industrial importance because any patient who has a history of alcoholism should not be exposed to this hazard. There is a peculiar feature about this association of alcoholism and carbon tetrachloride poisoning in that men who have that type of history tend to select this type of trade because they find they can get a cheap "drunk" with very little liquor.

(Dr. Moon) I believe there is a close relationship. I have looked at a great many kidneys specifically with that in mind, and there is cloudy swelling of the proximal convoluted tubules in the kidneys of both acute and chronic alcoholics. There are also, in some cases, binucleated epithelial cells and mitotic figures in the proximal convoluted tubules.

POST-MORTEM BIOCHEMICAL CHANGES. Walter W. Jetter and (by invitation) Regina McLean and Mary K. Nutter, Boston, Mass.

Abstract. A large portion of all medicolegal autopsies are performed on bodies of persons found dead and concerning whom there is little or no reliable anamnestic data. Every reliable source of information likely to shed light on the nature of the terminal illness or on the cause of death may need to be explored. An investigation of the chemical constitution of blood removed after death from a group of persons whose deaths were sudden and from causes least likely to have resulted in ante-mortem biochemical abnormalities was undertaken with two objectives. One was to assess the potential usefulness of such determinations in estimating the probable length of the post-mortem interval. The second was to determine the extent to which agonal or post-mortem changes might mimic biochemical abnormalities caused by disease.

Post-mortem samples of blood from the right heart were obtained from 20 persons. The interval between death and sampling varied from 1 to 69 hours. In all but 2 instances the bodies had been kept at refrigeration temperature for most of the post-mortem interval. In only one instance was there obvious putrefaction or hemolysis of the blood sample. Whole blood and plasma were analyzed for pH, carbon dioxide, glucose, non-protein nitrogen, and various electrolytes.

Certain changes in pH, lactic acid, plasma potassium, and inorganic and organic acid-soluble phosphorus were related to the length of the post-mortem interval. Generally speaking, the greatest change observed was that which had taken place during the agonal or early post-mortem period. All but one of the observed pH values were lower than any found in the living organism. The average for the first 12 hours was 6.79, while that for the second 12-hour period was 6.43. There was an irregular rise in plasma lactic acid during the first 12 hours. All values for plasma and cells were greatly elevated above normal. There was an immediate rise in plasma potassium which also increased irregularly with time. The cells showed a significant loss of this constituent. Inorganic phosphorus appeared in the cells at the same time that there was a great decrease of cellular organic acid-soluble phosphorus. Simultaneously the plasma contained a small amount of organic acid-

soluble phosphorus and a two-fold to nine-fold increase of inorganic phosphorus. The greatest increase was noted in the second 12-hour period. Plasma sodium was not significantly changed. The cells of 14 cases contained this electrolyte. Of the plasma calcium values, 11 were normal, while 5 were slightly elevated. Five cases showed significant invasion of the cells by calcium. Plasma magnesium was increased in 8 instances, decreased in one, and normal in the rest. Cell magnesium was increased. Cell chloride was generally increased, while in all but 3 cases plasma chloride remained normal or slightly decreased.

The carbon dioxide content of whole blood protected against loss of gas remained essentially normal. There was a substantial decrease in the carbon dioxide-combining power of plasma. A second series of cases showed the normal values for carbon dioxide content in whole blood to be due to greatly increased carbon dioxide tension. Non-protein nitrogen values varied between 28 and 124 mg. per cent. Glucose values ranged from 33 to 486 mg. per cent.

CHANGES OF EFFUSED BLOOD OUTSIDE AND INSIDE OF THE BODY: THEIR SIGNIFICANCE FOR LEGAL MEDICINE. George Strassmann, Waltham, Mass.

Abstract. After effusion of blood, changes in the hemoglobin occur outside or inside of the body. These changes were studied on human and animal material. If blood dries outside of the body, methemoglobin or hematin is formed after some time. In watery solutions the characteristic spectrum remains visible for weeks. The easiest method for identifying small and old traces of dried blood is the demonstration of hemochromogen crystals, which also give a characteristic spectrum. Crystals and spectrum of hemochromogen are similar in animal or human blood. Heating with a mixture of sodium hydroxide and pyridin gives good results.

Inside of the body phagocytic cells change the hemoglobin to hemosiderin and to hematoidin. After injections of homologous or heterologous blood in rabbits and mice, hemosiderin was found earlier than in spontaneous or traumatic cerebral hemorrhages of men. In many older cerebral hemorrhages of men or animals no hematoidin but only hemosiderin was discovered. The reason for the absence of hematoidin in such cases is doubtful. The presence of phagocytes with blood pigment is evidence that hemorrhage has occurred some time before death. The time interval between the onset of the hemorrhage and the first appearance of hemosiderin and hematoidin varies for different animals and men, and for different tissues and organs. In the brain the formation is slower than in other tissues. Perivascular phagocytes in the brain are indicative of a former hemorrhage which can be of traumatic or spontaneous origin. They are especially frequent in general paresis and cerebral arteriosclerosis. Phagocytes with the blood pigments remain in the hemorrhagic area for an indefinite period of months and years. In cerebral hemorrhages of man hemosiderin was not found before the 5th and 6th day, hematoidin not before the 11th to 14th day.

The stable combination of carbon monoxide with hemoglobin makes possible the detection of carbon monoxide in blood or bloody fluids from exhumed bodies in acute carbon monoxide poisonings, but outside of the body carbon monoxide-hemoglobin disassociates earlier and could not be detected in effused blood from fatal gas poisonings in animals after a few weeks.

* * *

INTRAMEDULLARY TUMORS OF THE SPINAL CORD AND FILUM TERMINALE. James W. Kernohan, Rochester, Minn.

Abstract. We have found in our series of 856 tumors of the spinal cord that approximately one-quarter arise from the spinal cord itself or the filum terminale. About 90 per cent of these lesions are gliomas, similar in most respects to gliomas

of the brain. In contrast to brain tumors, however, the majority of the intramedullary tumors are ependymomas, and in the filum terminale practically all tumors are ependymomas. The association of intramedullary tumors with syringomyelia is remarkably high.

Discussion

(Dr. Henry Edmonds, Washington, D.C.) May we hear from Dr. Kernohan concerning his ideas of the mechanism of syringomyelia in association with neoplasm?

(Dr. Bernard Black-Schaffer, Durham, N.C.) Since gliomas differ so markedly from the extra-nervous epithelial and mesothelial tumors, I should like Dr. Kernohan to comment further on his criteria for classifying the gliomas numerically as 1, 2, 3, 4.

(Dr. Kernohan) In regard to the first question, I am not sure that I can say what the association between syringomyelia and the neoplasm is. It is interesting that 62 per cent have associated tumor and syringomyelia. I rather think they are independent phenomena.

In regard to the question of malignancy, I have only about 3 minutes and it would take a little longer than that to explain this, but I did answer it somewhat on the lantern slides during my presentation. I pointed out that astrocytoma grade I is the same as the original astrocytomas described by Bailey and Cushing. The grade II astrocytoma has more cells, has pleomorphism and some hyperchromatic nuclei, but no giant cells, no mitotic figures, and the blood vessels are well formed. In astrocytoma grade III there is greater cellularity, more pleomorphism, some giant nuclei, and an occasional giant cell. There are only a few mitotic figures, only slight proliferation of the endothelial cells of blood vessels, and some proliferation of the adventitia of the blood vessels. In astrocytoma grade IV the tumor is very cellular, pleomorphism is marked, giant cells are usually numerous, and there are many mitotic figures. Proliferation of both the endothelial and adventitial cells of the blood vessels is marked and areas of necrosis are numerous. The same criteria apply to ependymomas and we have not yet evaluated our oligodendrogliomas. I hope this answers the second question.

EPIDERMOID CARCINOMA OF THE SKIN OF THE UPPER EXTREMITIES. Richard E. Johnson (by invitation) and Lauren V. Ackerman, St. Louis, Mo.

Abstract. Epidermoid carcinoma of the skin arising from the dorsum of the finger of the hand, or from the forearm, has individual characteristics and the rate of metastasis bears relation to certain pathologic findings. Seventy-two histologically proved consecutive tumors occurring in 71 patients were analyzed in detail. These patients were followed at least 3 years; they were outdoor workers (almost exclusively farmers) with histories of many years' exposure to the elements. In no instance was there an old scar, draining sinus, or arsenical dermatitis as an etiologic agent. The rate of metastasis in the entire group was 15 per cent, with an absolute 3-year cure rate of 59 per cent. The high death rate of 27 per cent during the 3-year follow-up period (without evidence of carcinoma) was attributed to the advanced age of the patients. Twenty-two per cent were over 80 and 49 per cent were over 75 years. The development of metastasis was contingent mainly on two factors: previous inadequate treatment, and the stage of the primary tumor. Of 24 previously treated tumors, 3 had metastasis on admission and 5 showed subsequent metastasis. Of 48 untreated tumors, 3 had metastasis on admission and none exhibited late metastasis.

Two distinct stages of tumor growth can be identified by the depth of invasion relative to the coiled portion of the sweat glands. All metastasizing tumors in

which the depth could be determined had extended below the sweat gland level. In the 24 previously treated tumors, 15 were below the level of the coiled portion of the sweat gland and 3 were indeterminate. Seven of the 15 and 1 of the group of 3 were found to have metastases. Of the 48 untreated tumors, 17 extended below the sweat gland level and 10 were indeterminate. Only 1 of these 17 and 2 of the group of 10 had metastases. Thus it is emphasized that previous inadequate therapy and depth of invasion are the most important factors in relation to the spread and prognosis in any group of these neoplasms.

A clear-cut division in two stages, above and below the coiled portion of the sweat gland, is easily explained. There is considerable resistance to invasion when the carcinoma meets the zone of degeneration of connective tissue fibers in the dermis. This layer of degenerating connective tissue in the dermis is consistently present, due perhaps to chronic exposure to sunlight, and natural ageing. In the presence of tumor it is progressively replaced by proliferating neoplasm and granulation tissue. This sequence of events results in a mass several times the volume of the original layer of degeneration. For a considerable period of time the growth capacity of the tumor is expended in replacing this area of degeneration and since the sweat glands arise just below this zone, they constitute an excellent manner of staging the tumor in terms of breeching of the "degeneration zone barriers."

INVOLVEMENT OF THE STOMACH IN MALIGNANT LYMPHOMA. H. R. Wahl and (by invitation) J. H. Hill, Kansas City, Kan.

Abstract. Five cases of malignant lymphoma in which necropy revealed unusual macroscopic infiltration of the stomach are presented. The infiltration, chiefly in the submucosa, produces two distinctive gross anatomic patterns. One is characterized by multiple discrete nodules with or without ulceration. The other is characterized by diffuse infiltration of the submucosa leading to the formation of giant rugae. In all 5 cases infiltration of the region of the antrum was minimal or absent. Except for fatal hemorrhages from the ulcerated nodules in a case of Hodgkin's sarcoma, no clinical signs or symptoms could be attributed to the extensive lymphomatous infiltration in these cases. It is believed these two gross anatomic patterns indicate involvement by malignant lymphoma.

Discussion

(Dr. Lauren V. Ackerman, St. Louis, Mo.) We have been impressed by the rather atypical clinical evolution of some of the lymphosarcomas of the stomach. In this group 5-year survival occurred rather frequently even with poorly conceived radiation and surgical therapy. I wonder if Dr. Hill would comment further on the evolution of lymphosarcoma of the stomach as compared with lymphosarcoma in other areas.

(Dr. William L. Robinson, Toronto, Canada) I was interested in the distribution of these lesions. They appear to be confined almost wholly to the fundic end of the stomach, and I wonder if Dr. Hill has made any attempt to localize these lesions, whether he has determined where the fundopyloric line is as compared to the localization of these lesions.

(Dr. Hill) We have in our material no satisfactory cases of primary lymphosarcoma of the stomach. I think this diagnosis is very difficult to establish. The surgeon may think he has a primary tumor only to find at necropsy that is not the case. Since we do not have such material, I cannot make any comments. I believe it may occur, but it must be very rare.

The distribution of the lesions in the stomach is of considerable interest. We do not understand why the infiltrations tend to occur in the fundus and body and not in the antrum. There are several cases of this same general type in the litera-

ture in which the prepyloric region is more extensively involved than the remainder of the stomach. Certainly the antrum contains lymphoid tissue and if these patients lived long enough I think they might all eventually have involvement of the antrum.

BENIGN LYMPHOID POLYPS OF THE RECTUM. Elson B. Helwig and (by invitation) James L. Hansen, Washington, D.C.

Abstract. Among the polypoid tumors of the rectum observed at the Army Institute of Pathology were 70 examples in which the lesion was comprised of lymphoid tissue. About one-half of these lesions were originally diagnosed microscopically as benign lymphoma, lymphoid polyp, heterotopic lymphoid tissue, and hyperplastic lymphoid tissue. The other half were diagnosed as giant follicle lymphoma, lymphosarcoma, malignant lymphoma, Hodgkin's disease, and reticulum cell sarcoma.

An analysis of the material contained in both groups showed a similar gross and histologic pattern. The lesions were polypoid, sometimes pedunculated and sometimes sessile, and generally, but by no means always, single. The surface was usually smooth and gray, but sometimes convoluted. The clinicians readily differentiated the lesion from a hemorrhoid, but often mistook it for an adenoma. The polypoid masses varied in size from 2 to 40 mm. Microscopically, the polyps were comprised of lymphoid tissue which exhibited a pattern of primary follicle formation with so-called germinal centers. The primary follicles as well as the germinal centers tended to be large and in many instances were of irregular shape. On the basis of the arrangement of the follicles a lobular structure was usually apparent, with the lobules of lymphoid tissue separated by a generally delicate connective tissue and reticular framework. Silver stain for reticulum showed a moderate amount of reticulum between the lobules of lymphoid tissue but only a sparse distribution within the lobules. Peripheral or central sinusoid structure, as found in normal lymph nodes, was absent. The lymphoid cells either in lobular formation or as ill-defined foci sometimes replaced the normal glandular mucosa.

General clinical examinations failed to disclose a single instance in which there were signs of any type of lymphoblastoma. Many of the examinations were particularly thorough because of the original diagnosis of lymphoblastoma. About one-third of these patients have been followed for several years and at this time none has developed generalized lymphoblastoma of any type. All but one patient, who received X-ray therapy, were treated by simple excision. On the basis of these observations polypoid lymphoid tumors of the rectum showing a pattern of primary follicles with germinal centers are to be considered benign. The structure of the lesions suggests that the change is one of hyperplasia.

Discussion

(Dr. Walter Putschar, Charleston, W. Va.) We have seen 4 cases of this type, in all of which the lesions were located at the mucocutaneous junction of the rectum. One is very much impressed by the features shown by the lesion which make one wonder if it is really a hyperplasia, or does it go back to the normal histologic structure which is exaggerated? It is a fact that some of the mammals, particularly the dog, have lymphoid tissue exactly in this area, and there may be a phylogenetic element in the formation of these lesions. All the lesions we have seen have remained benign, for as long as 11 years. All of them showed part of the squamous epithelium. None of the cases showed any inflammatory lesion of the rectum which would make inflammatory hyperplasia likely.

(Dr. Helwig) I am aware of what Dr. Putschar has pointed out, that in certain ruminants there is lymphoid tissue in the area of the mucocutaneous junction. In an attempt to see what type of inflammation might produce this lesion we

studied 45 cases of schistosomiasis, syphilis, lymphopathia venereum, tuberculosis, and of non-specific granulomas involving the rectum and anus, and in none was there an appreciable collection of lymphocytes. The one lesion in which occasional lymphoid follicle formation occurs is in apparently non-specific inflammation of the anal crypts. In this collection of lymphoid polyps we saw a portion of a crypt once in a while, but it seems doubtful that the entire group of lesions is associated with a non-specific cryptitis.

BENIGN LYMPHOMA OF RECTUM.* Elwyn L. Heller and (by invitation) Homer H. Lewis, Jr., Pittsburgh, Pa.

Abstract. The clinical and pathologic features of 9 cases of benign lymphoma of the rectum are presented, with a review of the appropriate literature. The importance of the lesion lies in the recognition of its benign characteristics in spite of histologic features simulating malignancy. In all probability the lesion is much more common than the occasional references would indicate.

Discussion

(Dr. Joseph E. Pritchard, Montreal, Canada) I would like to ask Dr. Heller if these tumors are ever multiple. They are usually solitary. All that I have seen have been strictly solitary. The question is important because I think sometimes the surgeon presumes there are other polyps there because of the polypoid nature of the tumor.

(Dr. Heller) In our series the majority of these cases were solitary, but multiple involvement has been described. I believe Dr. Helwig just stated that 60 per cent of his lesions were solitary, and I assume that 40 per cent were multiple.

A STUDY OF THE HISTOGENESIS OF ENDOCERVICAL METAPLASIA AND INTRA-EPITHELIAL CARCINOMA. Lee Howard (by invitation), Cyrus C. Erickson, and (by invitation) Leland Stoddard, Durham, N.C.

Abstract. The microscopic lesion of the cervix uteri that is known as intra-epithelial carcinoma, carcinoma *in situ*, or pre-invasive carcinoma, has been the subject of varied interpretation. Disagreement has concerned its site and mechanism of origin and its relation to invasive carcinoma. We think the available published data do not indicate clearly that this surface lesion inevitably becomes an invasive lesion. Continuing critical study and contributing observations are deemed preferable to unqualified acceptance of the inexorably malignant progression of this lesion. The present preliminary report records histologic observations on 400 total cervices and deals with the histogenesis of endocervical metaplasia and intra-epithelial carcinoma. Only 3 per cent were removed because of clinically observed cervical lesions; none was considered carcinoma. The mean age was 43 years. Only 6 pregnant uteri were present in the group. In each case, the entire cervix was cut into blocks and at least 2 sections made from each block so that 6 to 24 sections per case were examined.

Observations regarding the histogenesis of endocervical metaplasia agree with those of Carmichael and Jeaffreson and of Auerbach and Pund. Proliferation and differentiation of basal or reserve cells was followed from a thickness of one cell to the development of a complete surface layer. Such a differentiated, metaplastic lining could be distinguished from the normal squamous epithelium of the portio vaginalis. Some degree of metaplasia was present in 83 per cent of cervices. In an additional 1.8 per cent of cases, which included one of the 6 instances of pregnancy, cytologic atypicalities in metaplastic epithelium ranged from minor diver-

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

gence to extensive cytologic variation simulating intra-epithelial carcinoma. Intra-epithelial carcinoma was found in 3.5 per cent of cases.

It was possible to trace the proliferation of reserve cells through all stages of metaplasia to instances with cytologic variability and atypicality of sufficient degree to constitute the lesion termed intra-epithelial carcinoma. The great frequency of metaplasia compared with that of intra-epithelial carcinoma indicates that this progression is far from inevitable in a given case. The histogenesis explains the dominant location of intra-epithelial carcinoma, namely, the endocervical canal. In one case atypicalities extended onto the portio vaginalis, and, in a few instances not included in this report, lesions appearing in the portio vaginalis have been seen. The latter would seem to stem from a different histogenetic mechanism. No correlation of lesions with pregnancy or endocrine disturbances was possible. No instances of invasive carcinoma were uncovered in this study. Further observations concerning the potentiality of intra-epithelial carcinoma are required.

Discussion

(Dr. N. Chandler Foot, New York, N.Y.) The matter of intra-epithelial carcinoma is a very important one which is much to the fore at present. It is one which will need a great deal of study because in connection with the growing use of exfoliative cytology a great many intra-epithelial lesions are turning up. The question is, are these lesions reversible or are they not? We shall have to follow up all of these cases in order to answer that question. Whether they are reversible or not is difficult to say, but it is wise on the part of these authors to divide the intra-epithelial carcinomas and the metaplasias with atypicality. There is a dividing line there which is very tenuous, and it is difficult to say where one type goes over into the other. The cases of atypicality in the cervix with metaplasia will yield a great number of dyskeratotic cells which Papanicolaou calls "Birkhead" cells. These are always very difficult to interpret, and we find that in about half the cases they do not necessarily mean cancer, whereas in the other half intra-epithelial carcinoma is present. We must also be careful not to be didactic about intra-epithelial carcinoma and try to subdivide that one narrow classification into sub-heads, as some are doing at present. I think that that is going too far. In indicating the broadness of the subject I would like to point out that Bowen's disease of the skin, epidermoid carcinoma in the esophagus, and even carcinoma of the prostate show such intra-epithelial lesions, so that we must study a great deal more than these carcinomas of the cervix, and we shall have to follow them up very carefully in order to determine whether intra-epithelial carcinomas are true carcinomas that will necessarily go on to invasion. A great many of them do, and it seems likely that this is the very beginning of carcinoma, although we cannot be sure of it.

(Dr. Samuel R. Haythorn, Pittsburgh, Pa.) I have been interested in squamous cell metaplasia for a good many years, and I do not believe it is possible to take any set of lesions and trace squamous cell metaplasia into cancer. I think that there are two kinds of metaplasia, and one is primarily cancer from the beginning, and the other is a reversion to a lower type, or more protective type, of epithelium which is probably a part of a reparative process. While it is conceivable that the area might be involved with other tissues at the site of a cancer, it should not be considered a necessary step in cancer development, and in that sense it is reversible as Dr. Foot has said. Once this second type of metaplasia has occurred it does not follow that cancer is an irrevocable sequence.

(Dr. William L. Robinson, Toronto, Canada) We have been making a study of these cases also, and I was rather surprised that Dr. Howard and his co-work-

ers have not stressed one feature in metaplasia, the staining of the cytoplasm. I feel that carcinoma *in situ* gives a cell of more basophilic type as well as these other atypical characteristics, and as I was taught by Mallory, this and the loss of polarity of cells are the two distinguishing characteristics of malignancy. I would like to stress the basophilism of these cells.

(Dr. J. F. A. McManus, Birmingham, Ala.) Concerning the staining reaction of carcinoma *in situ*, the basophilia is apparently related to the increase in the ribonucleic acid in the cytoplasm. Dr. Findley and I have found that carcinoma *in situ* lacks the glycogen of the ordinary epithelium of the portio vaginalis, of the squamous variety. We have been interested in this matter of squamous metaplasia, repeating some of the work of Jeaffreson and Carmichael. We came to about the same conclusion as reported in this paper. Under certain conditions atypicality can appear in squamous metaplasia. There are metaplastic areas in which small droplets of mucin-like material are seen in the metaplastic cells of the endocervical glands, and others in which the mucin has disappeared. I am not sure that histologically one can suggest whether areas of squamous metaplasia are going to remain benign or become malignant.

(Dr. Joseph Tannenberg, Batavia, N.Y.) I like to emphasize in this connection the limitations of our morphologic observations. We have been concerned with these lesions for many years, and the more we see of them, the more we are compelled to state the impossibility in many cases of foreseeing whether these atypical cells will later become malignant tumor cells. There is only one thing to do: state what we see, and request observation of the patient in which such lesions are seen. We cannot foresee whether what appears to be metaplasia today will be a carcinoma in 6 months. We have to see new specimens eventually at monthly intervals, and repeated vaginal smears might be a helpful accessory in these cases. I would like to point out that we see the same lesions in caruncles and wherever squamous and columnar epithelium meet, if there is some kind of chronic inflammation. Thus I feel we have to emphasize the limitations of a single morphologic examination and have to see repeatedly, in 3 and 6 months, eventually, new biopsies from these patients if clinical examination shows suspicious lesions. I think we all have had occasion to see pitfalls of a single histologic examination when carcinomas have developed in patients who failed to see their physicians for a year or two after metaplastic lesions were seen, but hundreds of those patients will never develop carcinoma.

(Dr. Shields Warren, Boston, Mass.) This very careful study of Dr. Howard and his associates is the sort of thing I hope will be carried on widely. One of the chief values of the cytologic technic has been to emphasize the initial lesions that sometimes shed cells, and I believe that perhaps a great deal of the value of the method will be through the attention that it has called to these early and previously not too greatly noticed cervical lesions. One of the things that struck me in Dr. Howard's statistics is the same type of thing that we have found in our own statistics: the relatively high proportion of intra-epithelial carcinoma. That certainly is compatible with what Dr. Foot has pointed out as indicating a relationship with Bowen's disease of the skin. An incidence of 6 per cent runs far too high to permit this lesion to be a simple inexorable progression to carcinoma. I think it is one of the very real jobs of those interested in the cervical epithelium to attempt to evaluate in the next few years the abnormal epithelial lesions of the cervix which remain relatively static and those which progress to carcinoma. We have in Dr. Howard's series 3.5 per cent of intra-epithelial carcinomas, and I think it would be impossible to assume that these lesions are always precancerous unless some remain static or fairly static for long periods of time, or some of them re-

gress. Otherwise, we ought to have a tremendously greater incidence of cervical carcinoma in the female population than we have.

(Dr. Alfred Angrist, Jamaica, N.Y.) In this problem I would like to have the term "observation" defined. If these cases do have the possibility of developing malignancy, they survive a much longer period of time than the average early fully evolved carcinoma would suggest. Many of these may not go on to carcinoma in the ordinary sense. The term "observation" is what is variable and misleading. When this diagnosis comes forth from the laboratory, in some institutions the patient is immediately subjected to a major operative procedure. They do everything for this lesion from a radical Wertheim procedure, a panhysterectomy, or a simple hysterectomy to local excision, conization, or cauterization. How are we ever going to find out about the behavior of this lesion unless somebody has the courage to let these patients actually be observed to see what happens? I am anxious to know what you mean and recommend by the term "observation." Unless we have better standards as to the meaning of it, we will never know where we are at.

(Dr. Howard) I am afraid I will not be able to reply specifically to all of the questions and comments that were made. I am pleased that most of those discussing this paper would seem to agree in some respects with what I have said.

First, in regard to Dr. Foot's comments: I agree in believing that the follow-up in these patients is the important thing in developing what is the actual significance of this lesion.

In regard to the second comment, we feel that histogenetically the lesions we have shown are derived from the same cell, that is, the reserve cell. I think that in some instances the proliferation of this cell is perhaps along the lines of intra-epithelial carcinoma from the very beginning, and in other instances, it develops into what we call simple metaplasia, and perhaps stays there indefinitely.

In regard to the staining characteristics, I was not using color photomicrographs, but I have observed that in general the cytoplasm in metaplasia is of a pink character and in the intra-epithelial carcinomas it is of a more bluish color. We have not studied mucin preparations.

In regard to the follow-up, I think I can answer several of the questions at one time. By "observation" I mean follow-up of the individual case by repeated biopsies. These patients should be observed carefully and frequently by competent gynecologists. We have been fortunate in obtaining the cooperation of the gynecologic service and have been able to follow some of these patients over a long period of time. For example, we have seen several cases in which the lesion was present during pregnancy, and have followed the patient through three or four subsequent pregnancies with no change in the character of the lesion, so I think that the fundamental thing is to convince the gynecologists that the patients should be followed carefully and frequently by biopsy.

GENESIS OF CARCINOMA OF THE ENDOMETRIUM. Arthur T. Hertig and Sheldon C. Sommers (by invitation), Boston, Mass.

Abstract. A series of 400 cases of endometrial carcinoma has been reviewed in an effort to collect factors significant in the genesis of this neoplasm. Thirty-one cases were collected in which endometrial specimens had been taken from 1 to 23 years prior to diagnosis of carcinoma. Biopsy material taken 15 or more years before cancer diagnosis was normal, but at shorter intervals, biopsies were usually abnormal. The shortest interval between a normal curettage and carcinoma was 3 years. Cystic hyperplasia and endometrial polyps occurred in several cases, and were comparatively frequent 6 or more years before carcinoma. Adenomatous hy-

ers have not stressed one feature in metaplasia, the staining of the cytoplasm. I feel that carcinoma *in situ* gives a cell of more basophilic type as well as these other atypical characteristics, and as I was taught by Mallory, this and the loss of polarity of cells are the two distinguishing characteristics of malignancy. I would like to stress the basophilism of these cells.

(Dr. J. F. A. McManus, Birmingham, Ala.) Concerning the staining reaction of carcinoma *in situ*, the basophilia is apparently related to the increase in the ribonucleic acid in the cytoplasm. Dr. Findley and I have found that carcinoma *in situ* lacks the glycogen of the ordinary epithelium of the portio vaginalis, of the squamous variety. We have been interested in this matter of squamous metaplasia, repeating some of the work of Jeaffreson and Carmichael. We came to about the same conclusion as reported in this paper. Under certain conditions atypicality can appear in squamous metaplasia. There are metaplastic areas in which small droplets of mucin-like material are seen in the metaplastic cells of the endocervical glands, and others in which the mucin has disappeared. I am not sure that histologically one can suggest whether areas of squamous metaplasia are going to remain benign or become malignant.

(Dr. Joseph Tannenberg, Batavia, N.Y.) I like to emphasize in this connection the limitations of our morphologic observations. We have been concerned with these lesions for many years, and the more we see of them, the more we are compelled to state the impossibility in many cases of foreseeing whether these atypical cells will later become malignant tumor cells. There is only one thing to do: state what we see, and request observation of the patient in which such lesions are seen. We cannot foresee whether what appears to be metaplasia today will be a carcinoma in 6 months. We have to see new specimens eventually at monthly intervals, and repeated vaginal smears might be a helpful accessory in these cases. I would like to point out that we see the same lesions in caruncles and wherever squamous and columnar epithelium meet, if there is some kind of chronic inflammation. Thus I feel we have to emphasize the limitations of a single morphologic examination and have to see repeatedly, in 3 and 6 months, eventually, new biopsies from these patients if clinical examination shows suspicious lesions. I think we all have had occasion to see pitfalls of a single histologic examination when carcinomas have developed in patients who failed to see their physicians for a year or two after metaplastic lesions were seen, but hundreds of those patients will never develop carcinoma.

(Dr. Shields Warren, Boston, Mass.) This very careful study of Dr. Howard and his associates is the sort of thing I hope will be carried on widely. One of the chief values of the cytologic technic has been to emphasize the initial lesions that sometimes shed cells, and I believe that perhaps a great deal of the value of the method will be through the attention that it has called to these early and previously not too greatly noticed cervical lesions. One of the things that struck me in Dr. Howard's statistics is the same type of thing that we have found in our own statistics: the relatively high proportion of intra-epithelial carcinoma. That certainly is compatible with what Dr. Foot has pointed out as indicating a relationship with Bowen's disease of the skin. An incidence of 6 per cent runs far too high to permit this lesion to be a simple inexorable progression to carcinoma. I think it is one of the very real jobs of those interested in the cervical epithelium to attempt to evaluate in the next few years the abnormal epithelial lesions of the cervix which remain relatively static and those which progress to carcinoma. We have in Dr. Howard's series 3.5 per cent of intra-epithelial carcinomas, and I think it would be impossible to assume that these lesions are always precancerous unless some remain static or fairly static for long periods of time, or some of them re-

gress. Otherwise, we ought to have a tremendously greater incidence of cervical carcinoma in the female population than we have.

(Dr. Alfred Angrist, Jamaica, N.Y.) In this problem I would like to have the term "observation" defined. If these cases do have the possibility of developing malignancy, they survive a much longer period of time than the average early fully evolved carcinoma would suggest. Many of these may not go on to carcinoma in the ordinary sense. The term "observation" is what is variable and misleading. When this diagnosis comes forth from the laboratory, in some institutions the patient is immediately subjected to a major operative procedure. They do everything for this lesion from a radical Wertheim procedure, a panhysterectomy, or a simple hysterectomy to local excision, conization, or cauterization. How are we ever going to find out about the behavior of this lesion unless somebody has the courage to let these patients actually be observed to see what happens? I am anxious to know what you mean and recommend by the term "observation." Unless we have better standards as to the meaning of it, we will never know where we are at.

(Dr. Howard) I am afraid I will not be able to reply specifically to all of the questions and comments that were made. I am pleased that most of those discussing this paper would seem to agree in some respects with what I have said.

First, in regard to Dr. Foot's comments: I agree in believing that the follow-up in these patients is the important thing in developing what is the actual significance of this lesion.

In regard to the second comment, we feel that histogenetically the lesions we have shown are derived from the same cell, that is, the reserve cell. I think that in some instances the proliferation of this cell is perhaps along the lines of intra-epithelial carcinoma from the very beginning, and in other instances, it develops into what we call simple metaplasia, and perhaps stays there indefinitely.

In regard to the staining characteristics, I was not using color photomicrographs, but I have observed that in general the cytoplasm in metaplasia is of a pink character and in the intra-epithelial carcinomas it is of a more bluish color. We have not studied mucin preparations.

In regard to the follow-up, I think I can answer several of the questions at one time. By "observation" I mean follow-up of the individual case by repeated biopsies. These patients should be observed carefully and frequently by competent gynecologists. We have been fortunate in obtaining the cooperation of the gynecologic service and have been able to follow some of these patients over a long period of time. For example, we have seen several cases in which the lesion was present during pregnancy, and have followed the patient through three or four subsequent pregnancies with no change in the character of the lesion, so I think that the fundamental thing is to convince the gynecologists that the patients should be followed carefully and frequently by biopsy.

GENESIS OF CARCINOMA OF THE ENDOMETRIUM. Arthur T. Hertig and Sheldon C. Sommers (by invitation), Boston, Mass.

Abstract. A series of 400 cases of endometrial carcinoma has been reviewed in an effort to collect factors significant in the genesis of this neoplasm. Thirty-one cases were collected in which endometrial specimens had been taken from 1 to 23 years prior to diagnosis of carcinoma. Biopsy material taken 15 or more years before cancer diagnosis was normal, but at shorter intervals, biopsies were usually abnormal. The shortest interval between a normal curettage and carcinoma was 3 years. Cystic hyperplasia and endometrial polyps occurred in several cases, and were comparatively frequent 6 or more years before carcinoma. Adenomatous hy-

perplasia, that is, small outpouchings of endometrial glands with reduplication of lumina, and cellular anaplasia or de-differentiation, were relatively most frequent from 4 to 9 years before carcinoma. None of these changes are considered irreversibly neoplastic because of cases reverting to normal after ovulation. Carcinoma *in situ,* consisting of large pink cells with pale nuclei arranged in glands and often sharply demarcated, is the earliest lesion considered true neoplasia. Found up to 11 years before diagnosis of invasive carcinoma, it was relatively most frequently observed 4 to 5 years before the invasive stage. The 31 cases collected have essentially the same age distribution and mortality as found among the 400 cases, and are believed to form a representative group. They are unusual in that previous curettage in all 31 cases and radiation therapy in 19 of these cases did not prevent the development of cancer, since hyperplastic precancerous lesions presumably are more easily destroyed than invasive carcinoma.

Discussion

(Dr. Jacob Werne, New York, N.Y.) Those of us who see surgical pathologic material are asked what to do about the lesions presented here as precancerous. I wonder if the authors can tell what proportion of their cases showing these changes failed to develop carcinoma.

(Dr. Sommers) There were approximately an equal number of cases to the 32 with invasive carcinomas which in the final analysis we decided to discard from our series because of borderline or insufficient evidence of fully invasive carcinoma. The subject is being studied from the biopsy point of view. I would not be able to hazard a guess as to how frequently anaplasia and hyperplasia fail to go on to carcinoma.

HISTOCHEMICAL STUDIES ON ALKALINE AND ACID PHOSPHATASE IN RENAL CARCINOMA OF THE FROG. Gang Young (by invitation), Charles Breedis, and Balduin Lucké, Philadelphia, Pa.

Abstract. The common occurrence and availability of a variety of tumors in cold-blooded vertebrates permit investigation of their biochemical properties. Through such studies it may be hoped to broaden the scope of knowledge concerning the general characteristics of neoplasia. It was the aim of the present study to determine the activity of alkaline and acid phosphatase in relation to the development and growth of the carcinoma which commonly occur in the kidney of the leopard frog, *Rana pipiens.* The enzymes were chosen because they are known to be present in the normal kidney of the frog. Using a slightly modified method of Gomori, it was found that during the growth of the frog carcinoma, from minute localized nodules to large invasive and metastasizing tumors, profound changes occurred in enzyme content of the neoplastic cells. As the tumors became larger and more aggressive, alkaline phosphatase activity diminished and in many of the larger tumors disappeared entirely. In contrast, acid phosphatase activity increased until this enzyme became very abundant in the growing tumor. Metastases from the primary tumor, occurring relatively late in the course of neoplastic development, had a pattern of phosphatase distribution similar to that of the larger primary tumors. These results led to the conclusion that the activity of both acid and alkaline phosphatase becomes greatly altered, the former notably increasing and the latter decreasing during the course of development of the frog carcinoma.

Discussion

(Dr. Shields Warren, Boston, Mass.) I think it is of interest that a variety of tumors are shown in animals as well as in man to have this shift in the phosphatase property as the cells take on malignant tendencies. We have been interested in a

somewhat similar investigation of the early stages of carcinoma of the stomach in man, and there a somewhat similar shift of the relationship of alkaline and acid phosphatase appears to occur as the epithelium changes to a carcinomatous type.

HISTOCHEMICAL AND MICROCHEMICAL STUDIES OF RAT LIVER AFTER LOW PROTEIN DIET AND *p*-DIMETHYL-AMINO-AZOBENZENE. R. E. Stowell and (by invitation) C. S. Lee and K. K. Tsuboi, Kansas City, Kans.

Abstract. Three groups of experimental rats were given the following diets: (1) normal synthetic diet, (2) low protein, low choline, high fat synthetic diet, and (3) comparable low protein diet with the addition of *p*-dimethyl-amino-azobenzene. Animals from each group were fasted 18 hours and killed at intervals of 6 weeks, 3 months, and 5 months, and after tumor formation. Liver blocks were fixed by various methods and stained for alkaline phosphatase, lipase, nucleic acids, glycogen, lipids, ceroid, mitochondria, and collagenous tissue. The relative changes in cellular constituents were measured quantitatively by the Chalkley ratio method. Liver samples from these same individual rats were analyzed chemically for content of water, lipids, nucleic acids, alkaline phosphatase, esterase, succinic dehydrogenase, collagenous tissue, and nitrogen and phosphorus in various fractions.

Chemical methods of measuring collagen do not show high correlation with cytologic volumetric measurements. Volumetric ratio methods for visible lipids agree reasonably well with the chemical observations. The mean volume of the liver cell nucleoli enlarges two to five times in cirrhotic stages, the nuclear volume doubles, but cytoplasmic volume changes less. Ceroid stains with methyl green and gives positive reactions for carbohydrate components by the Hotchkiss technic and Feulgen reactions with, as well as without, acid hydrolysis. Total nucleic acids are increased in the cirrhotic livers, but some of the increase in the desoxyribose type may be attributed to the increase in fibroblast nuclei. Alkaline phosphatase reactions are variable, but esterase is decreased to one-half and succinic dehydrogenase to one-fourth in both groups with cirrhosis. The decrease in proportion of tissue that is liver cell cytoplasm may partially account for this decrease in total enzymatic activity. Regenerating cirrhotic nodules show increased histochemical lipase reaction. The presence of *p*-dimethyl-amino-azobenzene in a diet low in choline and protein reduces evidence of choline deficiency. In early stages there is less fatty metamorphosis, ceroid, and collagenous tissue formation in the liver. It is important to recognize the possibilities of such coordinated research in the proper interpretation of cellular physiology in terms of pathology, histochemistry and microchemistry.

Discussion

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder if the authors noticed any change in the degree of polymerization in their studies, *i.e,* if the size of the molecules of the nucleic acid fraction was studied from that standpoint, as by partial breakdown at different temperatures. In some work that was published we did find that there was a definite change in that direction, though total figures did not change as much as did the data obtained after partial hydrolysis or breakdown of polymers. This would indicate a definite difference in the size of the molecules in the hepatoma tumor fraction, in cirrhosis, and even in the liver tissue without the cirrhotic manifestation in animals on butter yellow. We found the same difference to occur in human livers with hepatoma and with cirrhosis.

(Dr. J. F. A. McManus, Birmingham, Ala.) There are several features of this study which appear extraordinarily interesting. One is this mosaic method of attack in which you have a number of technics giving different aspects of cellular metabolism, and another is the control of this study by comparison with the normal, which many of the histochemical reports appearing from time to time do not have.

In regard to the ceroid-like pigment, I think there are other pigments found in the tissues that have a carbohydrate component. Melanin under certain conditions does have some carbohydrate in it, and the same is true of the heart-failure pigment that we ordinarily call hemosiderin. There is a carbohydrate component in both of them.

I was interested in the histochemical demonstration of collagen. One of the technics needed most in histochemisty is something for protein. We have methods for carbohydrate and fat, but for the proteins, except the nucleoproteins, we do not have a satisfactory method. Some of the methods for amino acids determination may be used in the lower animals, but in the human being I do not know a satisfactory histochemical method for demonstrating protein. I was wondering which method Dr. Stowell is using for his collagen determinations.

(Dr. Paul Klemperer, New York, N.Y.) Is not the combined application of ultraviolet absorption spectrophotometry and controlled Feulgen reaction a definite indication for desoxyribosenucleic acid?

(Dr. Stowell) The study of the state of polymerization referred to in the first question would be interesting to investigate. Such an investigation was not undertaken because we were working with small samples, and we could not do everything with the samples used which in some instances were as small as 200 mg. of tissue. We hope we will eventually be able to take up other things of interest such as this.

In regard to Dr. McManus' comments, the chemical method for collagen used was that of Lowry, Gilligan, and Katersky (*J. Biol. Chem.*, 1941, 139, 795–804) as well as a comparison with the method of Spencer, Morgulus, and Wilder (*J. Biol. Chem.*, 1937, 120, 257–266). The first method was found to be more satisfactory from the chemical point of view, but we did not feel that either was as good as using a trichrome stain and the Chalkley ratio method for determining the volume of the collagen tissue. We hope that from this research we will get a better understanding of the relative advantages and limitations of various microchemical and histochemical methods. We are in the process of applying and evaluating the staining reactions for arginine, tryptophane, and tyrosin, and hope to get a reasonable indication of the distribution and amounts of these amino acids in tissues. In each of these animals the liver was separated into 4 fractions (acid soluble, alcohol-ether, nucleic acid, and protein) and the amount of nitrogen and phosphorus studied in each fraction.

In my remarks on ceroid I meant to show that ceroid gives reactions which might be easily misinterpreted as indicating that it contains desoxyribosenucleic acid if proper controls were not made. These histochemical technics are not so simple that you can read an article and go into the laboratory and blithely apply it to all kinds of tissue and assume you are measuring what you hope to detect. The Feulgen reaction when properly controlled is one of the best reactions we have for desoxyribosenucleic acid. Many people omit the proper controls. In studying nucleic acids by ultraviolet absorption I feel it is desirable to determine the absorption curve of tissues so one knows where the maximum absorption occurs. There are numerous substances which have an absorption at 260 mμ in common with nucleic acid, and I think it is hazardous to conclude that a photograph at one wave length constitutes a positive identification for nucleic acid.

THE EFFECTS OF A FOLIC ACID ANTAGONIST (AMINOPTERIN) ON ALBINO RATS. E. Woll (by invitation) and J. J. Oleson (by invitation), Burlington, Vt., and Pearl River, N.Y.

Abstract. Groups of rats, fed a stock diet, were given oral doses of aminopterin. The animals were sacrificed at frequent intervals, autopsied, and the body tissues

examined microscopically. Besides control groups, animals on a folic acid-free diet and aminopterin, and animals on water-free or starvation diets were similarly studied. The outstanding abnormalities were in the bone marrow and the gastrointestinal tract. The marrow at first showed a macrocytic and megaloblastic anemia which rapidly progressed to a total aplasia. The bowel changes were characterized by striking cellular degeneration, atrophy with cyst formation, and finally by acute ulceration and hemorrhage. The sequences of the above events were observed at frequent intervals.

Discussion

(Dr. Sidney Farber, Boston, Mass.) I want to congratulate the authors on these very interesting studies. The work of our own group in the past 2 years is in essential agreement with these findings. It is worth while mentioning that aminopterin and other similar anti-metabolites bring about essentially the same effects in mice, rats, dogs with leukemia, the normal dog, the man with leukemia, and the man without leukemia. I have great doubt as to whether these effects are produced as a simple folic acid deficiency. There is a growing body of evidence to show that these substances, while producing an anti-folic acid effect, act very much further than a simple antagonism. The effect on the bone marrow is of particular interest. This effect is not limited to aminopterin. Other compounds that are in the same group bring about equal changes. All of these amino-substances seem to have an effect on a wide variety of unrelated neoplasms, both in mice and in men.

(Dr. Woll) In our experience with the chick it was possible to reverse the morphologic change, that is, in consecutive animals, by giving them large doses of folic acid. We were not able to reverse the effects with folic acid in the rat. In this case my impression is that the alterations have gone beyond the stage where reversal was possible. We are apparently dealing with a deficiency which went on to the stage where it could not be reversed. The aplasia in the bone marrow was so severe that it was hard to see how the marrow could ever regain its normal activity.

THE TRANSPLANTATION OF EXPERIMENTALLY INDUCED BRAIN TUMORS. H. M. Zimmerman and (by invitation) Nelicia Maier, New York, N.Y.

Abstract. All the usual varieties of gliogenous tumors have been produced in several inbred strains of mice by the implantation of pellets of chemical carcinogens such as methylcholanthrene. Rather frequently the neoplasms display histologic features which vary in different zones. Thus, for example, a single brain tumor may consist of astrocytic, spongioblastic, and oligodendrocytic parts. Homologous subcutaneous transplantation of such "mixed" tumors has made is possible to separate them into their component parts and to establish "pure" lines of these neoplasms. Continued subtransplantation of the established lines has yielded samples of astrocytoma, ependymoma, oligodendroglioma, and polar spongioblastoma. The different tumors have become behavioristically stabilized in from 8 to 14 transplant generations so that the rate of growth of each is uniform and predictable. The constancy of behavior is paralleled by the microscopic appearance. In the case of glioblastoma multiforme, which is a heterogeneous neoplasm composed of many different cell types, it has proved possible to derive several "pure" tumors by the method of serial subcutaneous transplantation. From such a single tumor there have been derived an ependymoma, an astrocytoma, and an oligodendroglioma.

For future work with homogeneous strains of gliogenous neoplasms, the tumors developed in this laboratory will be of considerable value. Among other studies they will make possible those on the chemical composition and metabolism of brain tumors.

Discussion

(Dr. Russell L. Holman, New Orleans, La.) I infer that the tumors did not extend outside the central nervous system. Yet they apparently will grow outside the central nervous system. I wonder if Dr. Zimmerman would care to comment on this point.

(Dr. Zimmerman) We have undertaken as part of this study another one to determine just what Dr. Holman inquired about, namely, why neoplasms which have the ability to grow outside the nervous system—and we have grown these tumors in the anterior chamber of the eye, intraperitoneally, intrathoracically, and subcutaneously—do not metastasize spontaneously. This is also true of human neoplasms. The reason, I believe, is that none of these gliogenous neoplasms have the ability to invade blood vessels and therefore cannot get out of the cranial cavity. When we transplant them outside the cranial cavity they grow perfectly well. Inasmuch as the peripheral lymphatic system has no direct connection with the perivascular spaces of Virchow-Robin, there is no connection between the lymphatics of the intracranial and extracranial portions of the body, and so these tumors have no lymphatic pathway by which to get out of the brain. But within the cranium they will invade the leptomeninges, which are mesodermal tissues, and will grow upon the dura, which is mesodermal.

FACTORS AFFECTING THE DISTRIBUTION OF TUMOR METASTASES. Richard B. Eisenberg (by invitation), Erie, Pa.

Abstract. The concept that certain tissues afford an unsuitable "soil" for the growth of embolizing tumor cells has been presented repeatedly in the literature. For example, the failure of tumors to metastasize to the skeletal musculature has been attributed to physical and chemical factors unfavorable to the survival of embolizing cancer cells. Another possible explanation is that metastases are absent because cancer cells, owing to their mode of metastasis, rarely reach the skeletal muscle. The experiments to be reported were designed to determine the distribution of tumors in the body when tumor cell suspensions are artificially introduced into the arterial circulation. If the cells are capable of setting up growths in skeletal muscle under these conditions, it would appear likely that the "unfavorable soil" concept is erroneous and that absence of metastases in a part can be explained by failure of embolizing cells to reach the part. The tumor used for these experiments was a squamous cell carcinoma in rabbits (the V-2 carcinoma derived from the Shope virus papilloma). This tumor, maintained by serial transfer in domestic rabbits, metastasizes regularly to the adjacent lymph nodes, occasionally to the lungs, rarely elsewhere. This behavior is similar to that of squamous cell carcinomas in man. However, when in the present experiments suspensions of cells from this tumor were inoculated into the left ventricle, multiple metastases appeared throughout the animal, in the parenchymatous organs, skin, bone, central nervous system, and muscle. Cells injected into the femoral artery produced massive infiltration of the leg muscles with solid neoplastic growth. Injection of the femoral vein and of the right ventricle produced massive pulmonary metastases with no tumors elsewhere, indicating inability of the cells to pass the lungs and failure of the cells from the developing pulmonary tumors to gain entrance to the systemic arterial circulation. From these experiments it is concluded that absence of metastases in an organ is probably due to failure of the cells to reach the part, rather than to an unfavorable "soil" afforded by the part.

Discussion

(Dr. Shields Warren, Boston, Mass.) This is a very useful type of study. There is evidence that striated muscle forms a good substrate for the growth of a va-

riety of malignant tumors. Take carcinoma of the tongue, for example, which grows very rapidly into the muscle. I think there is one other point to be weighed, however. If one is using an emulsion of sieved cells, you will find frequently that these are not single cells, but are clusters of cells, and that if one uses single cells, as obtained by the method developed by Gates using ascitic fluid with the tumor cells growing therein, there is opportunity for the cells to pass through the coronary vessels, at least in the rat, and to reach other organs. Another point of importance is the aggregate number of cells required to establish metastasis. It is much easier for a cluster of four or five cells to establish a metastasis than it is for many times that number of single isolated cells to do so.

(Dr. Frederic Wohlwill, Hathorne, Mass.) It is also true in man that muscle tissue is not an unfavorable soil for the growing of carcinoma. When carcinoma invades a muscle locally it may grow not only between the muscle fibers, but within the muscle fibers, so muscle tissue should be a good soil for metastasis.

(Dr. Arthur Cloudman, Chicago, Ill.) Animals of certain genetic types show metastases in 100 per cent of their ovaries and kidneys in the case of one type of subcutaneously transplanted tumor, whereas the original host type gives very little evidence of metastasis to the ovaries and kidneys. There seems to be a tendency of certain tumors, such as a reticulo-endothelioma, to give a large number of metastases in some animals, and practically no metastases in other animals. I do not know how this may be explained; I think it is not explained merely on the basis of the mechanics of the circulation.

(Dr. John W. Harman, Madison, Wis.) There are several interesting points raised by this paper concerning the possibility of what I would call barriers to the spread of tumor. This possibility is not new, but was suggested by Martin Schmidt at the turn of the century as a result of his classical studies. Dr. Paulo Dacorso and I, in a study of a series of cases of generalized spread of carcinoma, have advanced evidence of such barriers, and in this presentation we find corroboration of our findings. We observed that in instances of generalized spread, once the tumor passed beyond the lung barrier, no organ was immune to metastasis and that both the pancreas and spleen, where secondaries are believed rare, contained metastases in 50 per cent of the cases. Obliteration of the barrier in the lung was accomplished in England by Foulds by means of injections of trypan blue. We reaffirm, strengthened by this evidence of the presented paper, that there are definite barriers within the tissues, determined mainly by mechanical factors such as impaction and coagulation, and that peculiarities of soil for growth and hormonal subtleties are not required to explain the spread of tumors.

(Dr. Eisenberg) Dr. Warren mentioned that passage of tumors through a sieve results in clusters of cells as well as single cells. We are unable to state the proportion of single cells to clusters of cells in the suspensions used in these experiments, nor have we information on the relative ability of emboli of these types to form metastases.

We are unable to explain the disparity of metastases in various organs, such as kidney and ovary, in host animals and transplanted tumors, as described by Dr. Cloudman. However, it is worth emphasizing that these experiments are preliminary, and, although no evidence for the "soil" hypothesis was disclosed, it is possible that fine degrees of variation in "soils" may be disclosed by quantitative methods in which varying known numbers of cells are injected into arteries supplying various organs.

We agree that the barrier to the passage of tumor cells through the lungs is probably mechanical.

HODGKIN'S DISEASE ASSOCIATED WITH MENINGO-ENCEPHALITIS. Antonio Rottino and (by invitation) George Hoffman, New York, N.Y.

Abstract. This report deals with a peculiar form of meningo-encephalitis which occurred in a patient suffering from Hodgkin's disease. The patient was a white male, aged 37, with onset of Hodgkin's disease in 1942 and diagnosed as such and treated with X-ray in 1944. In May, 1945, symptoms of encephalitis developed and he died 114 days later. Gross findings at autopsy were minimal. More significant ones were the microscopic findings, which showed involvement of the Virchow-Robin spaces throughout the brain and cord. The infiltrate consisted of cells resembling epithelioid cells, occasional multinucleated giant cells, and lymphocytes. In places these were arranged in tubercles. The conclusion reached was that the lesion was neither a Hodgkin's lesion nor a tuberculous lesion. It was sarcoid in character, and it was felt that it probably represented a phenomenon arising in response to changes which had occurred in a specific biologic mechanism and which had in this case been set into motion by the Hodgkin's disease process.

Discussion

(Dr. Leo Lowbeer, Tulsa, Okla.) In view of the fact that, as Forbus and his group have shown, certain types of inflammation due to brucella morphologically resemble Hodgkin's disease, I should like to ask whether an attempt was made to culture the meningeal exudate for brucella, and whether anything is known about agglutination titer and skin allergy for brucella in this patient. The morphologic character of the meningeal granuloma as demonstrated in the slides would be compatible with that found in brucellosis. Cases of brucellotic meningo-encephalitis with autopsy findings and microscopic examinations have been reported in the literature, by Hansmann and Schenken, Cull, Baggenstoss and Merritt, Scheidegger and Stern, Black, Noto, and Bergmarck, among others.

(Dr. Joseph E. Pritchard, Montreal, Canada) I would like to ask if syphilis was ruled out to Dr. Rottino's satisfaction.

(Dr. Rottino) In reply to the last question, there was no evidence of syphilis serologically. At autopsy no syphilitic lesions were found in the aorta or elsewhere.

In answer to the first question, brucellosis was not considered clinically. The picture clinically was that of encephalitis and attention was focused on it and on Hodgkin's disease. The usual bacteriologic studies were done and no organisms grown. No organisms were grown from the spinal fluid or demonstrated in the tissue sections.

HYPERSPLENISM IN MALIGNANT DISEASE—A NEW CONCEPT. Oscar B. Hunter, Jr., Washington, D.C.

Abstract. Observations of a case of lymphosarcoma treated with nitrogen mustard 2 years ago revealed a large spleen and the presence of a hyperplastic bone marrow. Laboratory studies during life revealed a rapid destruction of the transfused red cells and a consistently low white cell count. Post-mortem findings revealed almost complete absence of lymphosarcoma and the anatomic diagnosis was that of hypersplenism with severe anemia and leukopenia.

These findings have led to a laboratory study and correlation with post-mortem findings for a number of patients with various types of leukemia and lymphosarcoma. The essential findings are:

1. Splenomegaly due to leukemia or lymphosarcoma.
2. Hyperplasia of the normal elements of the bone marrow.
3. Exaggerated red cell destruction and/or diminution of the leukocytes and/or thrombocytes beyond what would be expected with the bone marrow findings.

4. Surgical and post-mortem studies of injected specimens reveal large dilated sinusoids lined by giant phagocytic cells containing nuclear débris and hemosiderin pigment.

5. Clinical follow-up of such patients has shown improvement following splenectomy.

The conclusions drawn from these findings are that in cases showing evidence of hypersplenism, even though due to a malignant disease, improvement can be expected from removal of the overly-phagocytic spleen. These findings are particularly applicable in some of the cases of acute leukemia treated with aminopterin where failure has resulted because of severe anemia or thrombocytopenia.

Discussion

(Dr. Joseph E. Porter, Portland, Me.) Dr. Hunter has emphasized the destruction of transfused red cells, and he has also cited a paper recently published in which hemolysis was demonstrated in lymphosarcoma and other allied diseases. I would like to know whether he has carried out Coombs' test on any of the cases investigated.

(Dr. Hunter) I regret that for the first 2 cases the Coombs' test (anti-human globulin test) was not carried out. In the last case, that of acute leukemia in which a splenectomy was done, this test was performed and was negative. It has been our experience in a series of 18 such cases that the Coombs' test is only occasionally positive and in the 2 cases in which we had observed a positive test the test has been open to considerable question. This particular state of hypersplenism I believe is not necessarily associated with hemolysins and in the cases observed we believe that splenic phagocytosis is the greater factor. This is well illustrated in the second case, that of a chronic lymphatic leukemia, which showed marked elevation of the red cell count and decrease in the required frequency of transfusions after splenectomy had been performed. Furthermore, platelet counts and white cell counts, when not influenced by medication as evidenced by the cases of lymphosarcoma, show a rise in both elements after splenectomy. From our observations of these cases we feel very definitely that the macrophages lining the splenic sinusoids have a great deal to do with the deficiency of the cellular elements in the peripheral blood.

STUDIES ON THE STRUCTURE OF ELASTIC TISSUE WITH THE ELECTRON MICROSCOPE. Jerome Gross (by invitation), Boston, Mass.

Abstract. Examination with the electron microscope of fragmented elastic tissue obtained from the aortas of man, rabbit and rat, bovine ligamentum nuchae, and fish swim-bladder revealed morphologically characteristic fibers differing markedly from collagen. Tryptic digestion of elastic tissue from all sources studied released large numbers of filaments, polydisperse in length, and fairly constant in diameter. A large proportion of these filaments appeared to be tightly coiled helixes composed of two intertwining threads. The distance between coils ranged between 500 and 650 Å in 100 measured filaments. The individual threads measured about 75 Å in diameter. pH was demonstrated to profoundly influence the physical state of the filaments.

These studies demonstrate the presence of two chemically and morphologically distinct components in elastic fibers. The relationship between structure and elasticity will be discussed.

Discussion

(Dr. Ernest W. Goodpasture, Nashville, Tenn.) I cannot forego the opportunity to express my personal appreciation to Dr. Gross for presenting this paper. Of course these newer methods are destined to play an enormous rôle in the fu-

ture in studies of morphology, and particularly morphology of the cell, in which we are all interested.

(Dr. H. Edward MacMahon, Boston, Mass.) I would like to ask Dr. Gross if he believes, from his studies, that collagen could be transformed into elastic tissue or if elastica could be transformed into collagen. The pictures that he has shown us of elastic fibrils as seen with the aid of an electron microscope are so completely different from those of collagen that it would not seem possible that these could be structurally interchangeable.

(Dr. Gross) Firstly, from amino acid analysis of decollagenated elastic tissue, one can see that the chemical pattern of elastin is very different from that of collagen. Elastin has somewhere near 90 per cent non-polar amino acids, whereas collagen has somewhere near 50 per cent, which would make a large difference in the organizational character and reactivity of the material; and secondly, from the fact that with the electron microscope you can see collagen fibrils passing into elastic fibers and yet the structure of the two is so different, you can imagine that on a histologic level you were seeing transitions of collagen into elastin, whereas this would actually be a passage of collagen bundles into the substance of an elastic fiber.

TISSUE RESPONSES TO PHYSICAL FORCES. IV. THE FIBROGENIC POTENTIAL OF FOREIGN PARTICLES IN TISSUE. Silas M. Evans (by invitation), Walter Zeit (by invitation), and Joseph J. Kuzma, Milwaukee, Wis.

Abstract. A variety of disease states are characterized by the development of fibrous tissue at the site of involvement. Oftentimes, these fibrous tissue reactions may be observed to develop in magnitude far beyond the apparent need of the situation. This apparent superfluity of fibrous tissue reactions is more striking as one considers that reactions of this type cannot be demonstrated to possess filtering or neutralizing properties to qualify them as defensive against solutions, toxins, poisons, or antigens.

The objections to the current solubility theory of silicosis are well known. Probably the most important objections appear to be that: (1) Some benign silicates provide the same ions in equal or greater concentration to the surrounding fluids as does quartz; (2) fibrous tissue is no better barrier against substances in solution than the tissue it replaced; and (3) it has never been shown that local concentrations of solution occur.

A consideration of the physical properties of fibrosive dusts reveals that, where data are established, fibrosive dusts belong to crystal classes which possess no point of molecular symmetry; whereas, benign dusts are of crystal classes with symmetric molecular structure or are non-crystalline (amorphous). Crystals which have no point of central symmetry will possess, if other conditions are proper, a property known as piezo-electricity. A piezo-electric crystal is one which is capable of transferring energy states in either direction between motion and electricity. When such a crystal is compressed mechanically, it induces an electric current; and, conversely, when it is placed in an electric current, it becomes distorted in shape. To illustrate, in phonographic reproduction, pressures induced upon a needle by the record groove are transformed through a piezo-electric crystal to the current which activates the speaker. The importance of the property of piezo-electricity in the biologic action of dusts has been explored in several ways. Rock quartz was physically reoriented to several different levels without chemical modification, and the fibrosiveness of the respective dust particles was observed. Previously untested non-siliceous ores were selected for their physical properties and fibrosiveness was correlated accordingly. The effects of physically induced energy releases within tissue were also observed.

Collagenous fibrous tissue is recognized to possess excellent tensile strength. In addition, preliminary study has indicated that this tissue, by comparison to other tissues, is highly non-conductive to heat and electricity. These physical properties qualify fibrous tissue as the best substance the body has to offer as a barrier or insulator against free energy in the form of motion, heat, or electricity. In view of these simple properties of fibrous tissue, it becomes imperative to reconsider the fibrogenic injury mechanism in those disease states hallmarked by active fibrous tissue proliferation. Such reactions would be functionally reconcilable if the injury could be demonstrated to be a release of abnormal energy within the affected part. In this case, energy in a form and magnitude sufficient to damage parenchymal cells would be localized and insulated against by the best qualified reactive tissue available to the host. The fibrogenic stimulus might then be considered to be energetic rather than material.

The fibrogenic potential of dusts is correlated with the piezo-electric activity of the dust.

Discussion

(Dr. H. Edward MacMahon, Boston, Mass.) Dr. Evans has just shown us that there is comparatively little response on the part of the body to diffuse amorphous quartz. I would now like to ask Dr. Evans if he believes it might be possible for the diffuse form of the quartz to recrystallize in the body and act as a strong fibrogenic agent. If crystallization can occur outside the body, there seems little reason why it should not take place within. I am thinking particularly of the disease, chronic beryllium pneumonitis. In this there may be a very long latent period between exposure and the disease process. Could an alteration in the physical state of the beryllium during this latent period account for the peculiar granulomatous changes in the lungs that are found in this process?

(Dr. Alan R. Moritz, Boston, Mass.) I think this is an extraordinarily interesting communication regarding a novel mechanism of physical injury. I was under the impression that adipose tissue was a poorer conductor of both heat and electricity than fibrous connective tissue. If that is the case and if the body were mobilizing its best defenses against electric or thermal injury, I should think it would lay down an adipose layer around the crystals rather than a fibrous one.

(Dr. Evans) In answer to Dr. MacMahon, vitrified silica can and indeed does revert to a crystal stage in many situations. The vitrified state is a thermodynamically unstable "high energy" state which is existent because of its high viscosity which resists molecular reorientation. Glass cutters will cut fresh glass at their own risk but refuse responsibility when asked to cut old glass. They know that with time, orientation and cleavage lines occur. We believe this mechanism to be one of the possible explanations for the fibrosiveness of amorphous materials.

In reply to Dr. Moritz, I can find very little on the physical properties of various tissues in the body. I think the work we have done with actual implants and measuring of resistances against alternating current has indicated to us that fibrous tissue is somewhat better than adipose tissue, which is paralleled by the fact that the body employs fibrous tissue as an insulating material where electropotential occurs physiologically as seen in the neurilemma.

PULMONARY LESIONS INDUCED IN WISTAR RATS BY URETHANE. F. K. Mostofi (by invitation) and C. D. Larsen (by invitation), Washington, D.C., and Bethesda, Md.

Abstract. Sixty Wistar rats, 5 to 8 weeks of age, about equally divided as to sex, were given single weekly intraperitoneal injections of a 10 per cent solution of urethane for 13 weeks (0.01 cc. per 1 gm. of body weight). At autopsy, 12 to 15 months later, more than one-third of the animals showed single or multiple pul-

monary lesions. The lesions were located peripherally, often subpleurally, were not elevated above the general surface level, and showed no relation to bronchi. Each consisted of a localized focus comprising several adjacent alveoli showing proliferation of alveolar cells in the stroma and within the alveoli. In 4 cases the alveolar lumina were virtually completely obliterated by a papillary cellular proliferation. There was no relation between this process and the bronchiectasis which is commonly seen in rats. The lesion was not found in control and other stock rats.

QUANTITATIVE STUDIES ON THE COLLATERAL CIRCULATION OF THE LUNG AFTER LIGATION OF THE PULMONARY ARTERY. William E. Bloomer and William Harrison (by invitation), Averill A. Liebow, and (by invitation) Milton R. Hales and Gustaf E. Lindskog, New Haven, Conn.

Abstract. By bronchospirometry and an application of the Fick principle to data obtained by bronchospirometry and analysis of the systemic arterial blood, the collateral circulation of the lung after ligation of the pulmonary artery was demonstrated to increase enormously. Most of the increase occurs within the first 2 weeks, but it is slowly progressive for at least 18 months. At the end of this time the collateral flow is approximately 1 l./m.^{2}/min.

The extent of the collateral bed demonstrated anatomically in vinylite corrosion casts and in histologic sections appears to be compatible with the magnitude of the flow as determined in the physiologic experiments. The bronchial arteries associated with the terminal bronchi now have a larger total area in cross section than the pulmonary arteries at the same level. This, together with the absence of morphologic changes in the walls of the alveoli, suggests that the peripheral resistance to blood flow in the lungs remains low.

The pulmonary artery does not become thrombosed distally, but comes into communication with the bronchial arteries through pre-capillary anastomoses that come to exceed 50 μ in diameter. Thus the pulmonary artery becomes a huge diverticulum of the bronchial arterial circulation and may, in part, absorb the impact of the systemic arterial pressure before the capillaries of the alveoli are reached. Under the conditions of the present experiments most of the blood entering the lung via the bronchial arteries is returned directly to the left auricle through the pulmonary veins. Thus the collateral circulation, which may exceed in volume one-third the output of the right ventricle, is a burden entirely upon the left side of the heart.

Discussion

(Dr. Jesse E. Edwards, Rochester, Minn.) This paper is of considerable interest. In a consideration of the differential pressures which must exist between the bronchial arteries and the pulmonary arteries under normal conditions and under conditions in which the bronchial arteries are dilated, the human being and some of the experimental material are not proper subjects for the measurement of such differences in pressures. It would seem that we might possibly have a means of doing this by cannulating the left pulmonary artery beyond the point of ligation and also the right pulmonary artery, to determine whether there exists a differential pressure between the two lungs. I should like to ask Dr. Liebow whether he has any evidence concerning the pressure in the lung which is receiving its blood by way of the normal pulmonary circuit on the one hand, and the lung which is receiving its circulation through the collateral system derived from the aorta on the other.

(Dr. Liebow) In answer to Dr. Edwards' question, we had not intended to go into this problem at this time because we do not have a great deal of evidence on the point in regard to the dogs. In the case of chronic, severe, unilateral bron-

chiectasis in man, however, before any operative procedure is done the two pulmonary arteries can with good fortune be entered separately by means of catheters. When that is done the pressure on the diseased side, as determined by Hamilton manometry, is considerably in excess of the pressure in the relatively normal side. This suggests that blood may be flowing backwards in the pulmonary artery of the diseased side toward the hilum of the lung. That is rather easy to understand because of the large size of the anastomoses between the two systems and the fact that the blood in the expanded bronchial arteries is under high pressure.

One additional proof is necessary—to determine whether the blood in the pulmonary artery on the side of disease has the same characteristics as the blood in the femoral artery. That is, indeed, the case.

THE PATHOGENESIS OF EXPERIMENTAL FAT EMBOLISM. John W. Harman, and Florian J. Ragatz (by invitation), Madison, Wis.

Abstract. Fat embolism was produced in rabbits by the intravenous injection of homologous liquid fat, obtained by alcohol extraction of perinephric fat. The volume of fat injected was proportional to body weight and the lethality of the dose thereby determined. It was found that the mortality rates for 0.45, 0.55, 0.75, and 0.90 ml. of fat per kg. of body weight are, respectively, 16.5, 50, 66, and 100 per cent. Dosage *per se* has a decided effect upon the mortality rate, whereas rate of injection and repetition have slight effect. With a constant dose the effect of other factors upon mortality can be examined.

A series of rabbits was studied for the influence of trauma on the mortality due to fat. Trauma was instituted by the application of a tourniquet to one thigh so as to occlude the blood supply completely for 4 hours. An hour after release of the tourniquet fat was injected, 0.45 ml. per kg. intravenously. The mortality rate was 41 per cent in contrast to 16.6 per cent for the fat-injected controls and 0 per cent for the 60 traumatized controls. Because of the considerable fluid loss into the traumatized limb, another series of rabbits was dehydrated by water deprivation and injection of hypertonic glucose. These suffered considerable weight loss and some hemoconcentration. The injection of 0.45 ml. per kg. of fat produced a 50 per cent mortality with extremely rapid death as compared with the controls. This suggests that dehydration may be a factor in the deaths due to trauma combined with embolism.

Both gross and histologic studies revealed the principal changes to be in the lungs, which were very edematous, studded with minute areas of hemorrhage, and infiltrated with polymorphonuclear leukocytes. Animals with the most extensive pulmonary lesions often had a widespread centrolobular coagulation necrosis of the liver cords. Ascites and pleural effusion were also encountered. These lesions suggested anoxic anoxia as the chief disturbance. To test this, a series of rabbits injected with 0.55 ml. per kg. of fat were maintained in an atmosphere of 80 per cent oxygen for a period exceeding the survival period of untreated fat-injected controls. This therapy reduced the mortality from 50 per cent to nil, with slight alteration of the pulmonary lesion, but obviation of hepatic changes. The pulmonary alteration was qualitatively similar to the controls, though less extensive. In a small additional group of rabbits injection of paraffin oil was characterized by a higher mortality as compared with homologous fat. The action appeared mechanical, however, because the lungs contained no lesions or edema, such as was seen with the fat injections.

It may be concluded, therefore, that fat in higher doses may cause death by mechanical obstruction, and in low doses damages the pulmonary intimate vasculature. The damage results in stasis, edema, and anoxic anoxia, which may cause

death. This may be prevented by adequate oxygen therapy, although the development of the lesion is unaffected. On the other hand, both dehydration and tourniquet shock augment the mortality significantly. It is suggested that the trauma acts by dehydration associated with fluid loss into the traumatized area.

Discussion

(Dr. O. J. Pollak, Quincy, Mass.) I would like to ask two questions: 1, as to the size of the fat globules injected, 2, as to the presence of fat in cells of the subintimal layer and the fat stains used.

(Dr. Harman) The size of the fat globules may be determined roughly by watching the fluid break up as it enters the blood stream, but the injection was at such a rate that we could not observe their exact size. We made no attempt to produce an emulsion or suspension of uniform particles.

As to the stains for fat, we did extensive fat stains on all of the tissues, and particularly stained the tissues in which necrosis occurred. We do not believe that the use of the Nile sulfate stain is applicable or reliable. We were especially interested in the fat in the liver and tried to exclude the possibility of fat emboli passing into the peripheral portion of the lobule and producing central ischemic necrosis. We did not find either paraffin or neutral fat under the circumstances. The subintimal cells of pulmonary arteries, so far as we could determine by means of sudan III stains, did not contain demonstrable fat. We presume that the swelling of these cells, therefore, is due to the imbibition of fluid other than fat.

BONE CHANGES OBSERVED FOLLOWING INTRAVENOUS INJECTIONS OF BERYLLIUM. A. M. Cloudman, Dorothy Vining, S. Barkulis, and J. J. Nickson (all by invitation), Chicago, Ill.

Abstract. It was reported several years ago that intravenous injections of insoluble beryllium compounds can produce bone tumors in rabbits (L. U. Gardner and H. F. Heslington, *Federation Proc.*, 1946, **5**, 221). The work reported here was started to confirm the above and to extend data to include mice.

The animals were arranged in groups called I, II, III, and IV, which received zinc beryllium silicate, zinc silicate, beryllium oxide, and no treatment, respectively. The rabbits were injected through the ear veins and the mice received tail vein injections. Semi-weekly injections were made until all animals had received a series of 20 to 22 injections. The group I rabbits each received a total of about 550 mg. of Zn and 17 mg. of Be; the mice in this group each received about 8.36 mg. of Zn and 0.264 mg. of Be. In group II the total dosage to each rabbit was about 390 mg. of Zn while each mouse received about 2.8 mg. of Zn. The beryllium oxide used in group III was substantially 100 per cent BeO by spectrographic standards. The rabbits in group III received a total of about 390 mg. each and the mice received 1.54 mg. each.

In the rabbits bone changes were followed by means of periodic X-ray films of the entire skeleton. Terminal X-ray films were taken of the mice. The rabbit films showed that only group I animals, or those receiving $ZnBeSiO_3$, showed bone changes. Irregular thickenings of the cortices of the long bones were observed as early as 94 days after injections were started. These changes were most evident in the humerus, femur, and tibia. The bones of the pelvis and skull also showed some increase in density. This thickening did not proceed at the same rate in all rabbits of group I, yet 4 of 5 animals that lived over 1 year from the start of the injections have developed bone tumors. All but one of these bone tumors showed gross evidence of metastases. Group I mice also showed some bone tumors that were malignant. This is not true to date of the mice in the other groups.

Discussion

(Dr. Russell L. Holman, New Orleans, La.) I should like to ask what form of beryllium was used, what compounds or "trade names" we should be on the lookout for, and if Dr. Cloudman knows any compounds other than beryllium which might cause such bone changes.

(Dr. Frank Dutra, Cincinnati, Ohio) I would like to state that pure beryllium oxide, administered intravenously, has been found capable of producing tumors in rabbits, in the same way that these tumors occurred in the animals discussed here. The carcinogenic activity does not depend upon the zinc and silica of the substance. We were able to induce almost exactly the same number of tumors using pure beryllium oxide as with zinc beryllium silicate.

I would like to comment on the possibility of tumors due to beryllium occurring in man. We have been interested in this, and we are convinced that no such tumors have occurred to date. The amount of beryllium administered to these animals, per unit of body weight, is far greater than any human is likely to have gotten in industry. The amounts recovered from the tissues of our rabbits with tumors far exceed the quantities recovered from any employee who died of either acute or chronic pulmonary berylliosis. It may be that the route of administration is highly important in determining carcinogenic activity; I would be delighted to have Dr. Cloudman's comments on this.

(Dr. Cloudman) I do not know of any non-radioactive elements other than beryllium that produce bone tumors. Some of you may know of some, but I do not.

As for the hazards, these rabbits got 17 mg. of beryllium element, that is, they got about a gram of the zinc beryllium silicate, and many of the rabbits that got the tumors first had less than that. Just how much danger there is of getting bone tumors in industry I do not know. This is the result of intravenous injection, and yet I think that anything that can result from beryllium should be investigated and reported upon. I know that some investigators have produced bone tumors with beryllium oxide, and we were unable to do that, although I must say that our animals receiving beryllium oxide did not survive as long as I would like to have had them survive. The minority survived a year, but I had X-rays of the bones that showed no changes in the terminal state.

HISTORADIOGRAPHY—A STUDY OF CHEMICAL ELEMENTS IN HISTOLOGIC SECTIONS BY MEANS OF X-RAY ABSORPTION. Leonidas Marinelli (by invitation), Gaetano Ferlazzo (by invitation), Patrick J. Fitzgerald, and Frank W. Foote, New York, N.Y.

Abstract. Radiography is based on the fact that X-rays are absorbed by matter and that the amount of absorption is dependent upon the wave-length of the X-rays as well as on the density and atomic number of the absorbing material. Industry has utilized these facts in the soft X-ray region to investigate the fine structure of metals and alloys. Soft X-rays have been applied to biologic studies such as insect anatomy, and the structure of plant and animal life. Lamarque and others have used this technic with histologic sections and obtained differences in X-ray absorption by various substances in skin, thyroid, cartilage, and blood vessels.

It is well known that the absorption of X-rays by a chemical element will change abruptly at the wave length which is specific for the chemical element in question. Such discontinuity (absorption edge) permits the identification of an element in the presence of others, if proper technics are employed. A few elements have been identified and measured in histologic sections by Engström (calcium in blood

vessels, calcium and phosphorus in dentine of teeth, and sulfur in skin). The latter states that extremely small amounts of these elements, 10^{-10} gm. or less, can be determined in a tissue area of about 100 μ^2 with an estimated analytic error of 10 per cent. The physics department at Memorial Hospital has developed a technic whereby routine paraffin-embedded sections of surgical specimens of human or animal thyroid gland, 10 μ thick, can be studied for their iodine content keeping the tissue intact so that the histologic detail can be correlated with the characteristic absorption effect. The paraffin section is floated onto a photographic plate, dried, and with the tissue overlying the photographic emulsion, both are exposed to an X-ray beam that is preferentially absorbed by the element iodine. Areas that contain colloid in normal thyroid glands absorb these X-rays and no reduction of the underlying photographic emulsion occurs. All areas other than colloid allow the passage of X-rays which on reaching the photographic emulsion reduce it. When the photographic plate is processed, the areas of absorption (containing colloid and presumably iodine) are light. The non-absorbing areas are dark. Correlation of the histologic detail with light absorption can be made. Since most of the X-rays from our tube are of a wave length close to the L absorption edge of iodine (2.7 Å), we believe that we have demonstrated iodine in the sections presented.

We have studied about 600 historadiograms in the development of the technic. Normal human thyroid showed absorption in the colloid of the follicles. Follicles differed considerably in amounts of absorption and even adjacent follicles varied widely. Papillary adenocarcinoma of the thyroid revealed no absorption in papillary areas but a few intact thyroid follicles in the same section showed absorption. Follicular and alveolar adenocarcinoma demonstrated absorption of varying degrees where colloid was present.

Theoretically, it is possible to apply the method of historadiography to the localization of all chemical elements other than hydrogen; however, many technical difficulties are anticipated in the identification of elements of very low atomic number.

ANTIGEN TRACER STUDIES AND HISTOLOGIC OBSERVATIONS IN ANAPHYLACTIC SHOCK IN THE GUINEA-PIG (PART II). Frank Dixon (by invitation) and Shields Warren, Boston, Mass.

Abstract. Crystalline egg albumen combined with a radioactive isotope of iodine, I^{131}, was used as a labelled antigen in the study of anaphylaxis in guinea-pigs. Three groups of animals were studied: the first, sensitized with non-radioactive, iodinated albumen and shocked with radioactive, iodinated albumen; the second, non-sensitized controls, injected with a single dose of radioactive albumen; the third, sensitized and shocked with fibrinogen and with an injection of radioactive albumen made simultaneously with the shocking dose of fibrinogen. The radioactive albumen was traced in the tissues of these animals by means of Geiger counts and autoradiograms.

The most significant difference between the antigen distribution in shocked animals (group I) and non-sensitized controls (group II) was in the lungs. Shocked lungs contained more than one and one-half times as much antigen as lungs of controls. Autoradiograms showed the increased amount of antigen to be in the edematous collagenous tissue of the bronchial walls and of the adventitia of pulmonary vessels. This collagenous tissue in the controls was not edematous and contained no antigen.

To determine whether this localization of antigen about the bronchi and pulmonary vessels in anaphylaxis was a function of the specific antigen or the result of a non-specific leakage of protein through permeable vessels, fibrinogen was used

as the sensitizing and shocking antigen in the third group of guinea-pigs, and labelled albumen was given simultaneously with the shock dose. The labelled albumen content in the tissues of these animals closely approximated that in the non-sensitized controls. The amount of antigen in the lungs was not increased over that in the controls, and only a small amount could be demonstrated in the bronchial and vascular walls. This suggests that the localization of the antigen in the edematous collagenous tissue of the bronchial and vascular walls in anaphylactic shock may be a specific function of the causal antigen.

The mechanism of bronchial obstruction in albumen anaphylaxis which terminated fatally in 2 minutes was somewhat different from that observed in the previously reported globulin anaphylaxis which terminated fatally in from 5 to 8 minutes. The rapidly fatal obstruction in albumen shock resulted from a combination of smooth muscle contraction seen around a few of the bronchi and edema in most of the bronchial walls. In the more prolonged globulin shock, smooth muscle contraction occurring in the first 1 to 3 minutes had disappeared by the time of death, and the only significant finding was extensive edema in the walls of almost all of the medium and large bronchi. It thus seems likely that smooth muscle spasm is a transitory phenomenon in anaphylaxis and is most important during the first few minutes of shock. Edema, on the other hand, apparently increases, becoming a progressively greater factor in bronchial obstruction in prolonged shock.

Discussion

(Dr. Jerome Gross, Boston, Mass.) Would this radioactive antigen tracer technic demonstrate which components of connective tissue are involved in this process?

(Dr. Dixon) This method is not as yet sufficiently refined to localize radioactive materials of this type to individual cells or individual fibers. About the best we can do is to localize it to individual areas, such as the peripheral parts of the bronchial wall.

SERIAL BIOPSY OF THE TESTIS: OBSERVATIONS IN ENDOCRINE DYSFUNCTION AND STERILITY. Ronald C. Sniffen (by invitation), Boston, Mass.

Abstract. Testicular biopsy was employed in patients with various types of hypogonadism as an aid to diagnosis and the evaluation of therapeutic measures. At the close of the series over 300 men and boys had been studied. The patients fell into two large groups: (a) apparently sterile individuals without detectable endocrine dysfunction, but with oligospermia or azoospermia; and (b) those with obvious endocrine disorders.

In the first group approximately half the patients showed abnormalities of spermatogenesis in the form of either hypospermatogenesis, arrest of spermatogenesis, or complete absence of germ cells. The other elements of the tubules were not affected. The other half of the group showed an essentially normal gland, and at operation were found to have congenital or acquired defects in the excretory duct system. In the absence of endocrine dysfunction, definite abnormalities in the interstitial cells were not observed. Hormone therapy had no appreciable effect on spermatogenesis in this group.

In this presentation only patients with the following abnormal endocrine manifestations are considered: (a) eunuchoidism with low follicle-stimulating hormone (FSH) values, (b) patients with hypophyseal injury, and (c) precocious boys. It was found that in patients with eunuchoidism with low FSH values and an immature testis, testosterone produced tubular maturation and stimulated spermatocytogenesis, but had no noticeable effect on the interstitial tissue. APL, on the other hand, led to Leydig cell development and tubular maturation with stimula-

tion of spermatocytogenesis. Both forms of therapy resulted in the return to normal of a tunica propria previously showing collagenous thickening.

In the presence of an intracranial lesion that damaged the hypophysis the testicular picture varied with the age of onset and the severity of the damage. If the onset occurred before puberty and resulted in hypopituitarism, the testis remained in an immature state. If the hypophysis was injured after puberty, the testis showed regressive changes that seemed to be characteristic of hypophyseal suppression. The features of this regression consisted of hypospermatogenesis and finally aspermatogenesis, and accumulation of fatty substances in the Sertoli cell cytoplasm, collagen and elastic tissue deposition in the tunica propria, and gradual atrophy of the Leydig cells. In a series of testes from men with carcinoma of the prostate treated with stilbestrol for varying intervals before orchiectomy, the tubules and Leydig cells followed the same path of regression as in organic hypophyseal suppression.

Precocity in children is of two main types: (a) "central" precocity, where the stimulus presumably arises in the hypothalamus or hypophysis, and (b) "adrenal" precocity due to hyperplasia or carcinoma of the adrenal cortex. Testicular biopsy in a small group of children indicated that in "central" precocity both tubules and Leydig cells are stimulated, while in "adrenal" precocity the tubules mature but there is no stimulation of spermatogenesis or Leydig cell formation.

TISSUE REACTIONS TO SUBCUTANEOUS IMPLANTS OF THERMALLY DENATURED PORCINE SKIN. Richard Ford, Boston, Mass.

Abstract. The observation that severe human burns are subject to unpredictable rates and types of reparative processes has led to the following study of the denaturation of skin by controlled *in vitro* heating. Using a controlled-temperature, circulating bath of water or mineral oil of ± 0.1° C. accuracy, segments (approximately 5 by 7 mm.) of freshly excised skin from weanling domestic hogs were heated inside thin synthetic rubber sheaths at various temperatures from 45° to 125° C. The heated skin was then implanted in subcutaneous pockets in the donor hog for periods of about 4 weeks.

An initial series heated for 10 minutes after the tissue reached the bath temperature and implanted for 25 days gave the following results: At 40° and 50° C. epidermis survived and grew upon the original dermis or upon new stroma without rete pegs. The implanted dermis proliferated marginally and merged with the fibrous tissue of the surrounding area. At 53° and 54° C. no epithelium survived and the dermis was broken up and replaced by invading strands of new fibrous tissue with some inflammatory exudate. Scavenger cells, including foreign body giant cells, were effectively removing traces of dead epithelium, hair, and cornified layers. Between 55° and 90° C. the dermis was encapsulated by a thin zone of new fibrous tissue. The dead epithelium, hair, and cornified layers were again effectively subject to scavenger cell action. At 100°, 115°, and 125° C. the dermis and epidermis were surrounded by a wide zone of coarse fibrosis infiltrated by an exudate predominantly neutrophilic. The exudate had invaded the heated dermis without concomitant fibrosis and masses of leukocytes appeared to have replaced the dead dermis by lysis. The dead epithelium and appendages evoked moderate foreign body scavenger reaction.

Two additional series were implanted after being brought to the desired temperature and then immediately cooled in a room temperature water bath. Tissue temperature was recorded by wrapping the sub-dermal face of the tissue around the thermocouple tip and tying with thread. In one series, time of heating from 50° C. to the desired temperature and back to 50° C. was recorded in each case and it varied from 38 seconds for 51° C. to 147 seconds for 70° C. Epithelium

survived on the original dermis with persistent rete pegs at 50°, 51°, and 52° C. At 53° and 54° C., also, epithelium persisted but appeared only on a new flat stroma and was pale-staining, flattened, and relatively less cellular. At 53° and 54° C. the implanted dermis appeared dead in that it was non-proliferating and vascularized by capillaries surrounded by new connective tissue. From 55° through 70° C. epidermis and dermis were dead. There was slight encapsulation of the dermis without vascularization, fibrosis, or exudative reaction of the dermal mass. The epithelial detritus evoked marked scavenger cell reaction.

In these three series epidermis was killed by heat at 55° C. and dermis at a slightly lower temperature when heating was brief. As in the case of certain other forms of noxious radiant energy, heat appears to injure the dermis sooner than the epidermis. Dermis was heat-fixed above 55° C. and remained unaltered until 100° C. Above 100° C. it became subject to lysis and infiltration by inflammatory cells. Nearly lethal temperatures appear to denature the living epidermal epithelium.

Discussion

(Dr. Shields Warren, Boston, Mass.) I would like to ask if there is any parallel study as to the change of the proteins of the porcine skin which might give a clue to this variation in reaction at the two temperatures you have described.

(Dr. Ford) I understand that biologists of the Massachusetts Institute of Technology have been working on this matter, but I am not familiar with this phase of their work. I feel that the problem from my own angle is still incomplete.

HISTOPATHOGENESIS OF CARCINOMA OF THE FORESTOMACH OF MICE INDUCED BY INTRAMURAL INJECTION OF 20-METHYLCHOLANTHRENE. H. I. Firminger (by invitation) and H. L. Stewart, Bethesda, Md.

Abstract. An attempt was made to characterize early neoplastic changes in stratified squamous epithelium by studying the histopathogenesis of squamous cell carcinoma of the forestomach in mice. One one-hundredth cc. of an aqueous methocel suspension containing 30 mg. of methylcholanthrene per cc. was injected into the wall of the forestomach of 200 young adult mice of strains C_{57} brown and A backcross, equally divided as to sex. They were killed on the average of 4 per week for 12 months.

Histologically, within the first 3 days there was hyperkeratosis, acanthosis, mucosal edema and irregularity of basal cells overlying the injection site. There was a reaction about the methylcholanthrene in the submucosa characterized by edema, inflammatory change, necrosis, and degeneration of collagen. Subsequently, the mucosa showed various abnormal hyperplastic and dyskeratotic changes. The basal layer thickened progressively, its cells became irregular in shape, staining, and orientation and formed small buds. In occasional foci the basal cells were lost owing to degeneration, leaving the spinous cells in direct contact with the lamina propria in that area. Several retia showed inversion of keratinization at their growing tips. Disorder of keratinization was reflected by pre-eleidin granules or single cell keratinization even in the basal layer, and by abnormality of the granular layer, parakeratosis, and "corps ronds" formation. Squamous cell carcinoma occurred first at 39 days and was frequent after 2 months. Sarcomas were infrequent and did not occur until 91 days or more after injection.

THE INFLUENCE OF PHOSPHORUS UPON THE ABSORPTION OF IRON. T. D. Kinney and (by invitation) D. M. Hegsted and C. Fitch, Cleveland, Ohio, and Boston, Mass.

Abstract. It has been shown that rats fed a diet of 80 per cent corn grits and 20 per cent lard plus haliver oil absorbed and deposited in the liver large amounts

of iron when excessive amounts of ferric citrate were added to the diet. Animals fed control diets of purina dog chow containing the same amount of ferric citrate showed an insignificant increase in the liver iron. This increase in iron in the livers was not due to a redistribution of body iron following the loss of body weight, since animals fed the corn grit diet without added iron lost comparable amounts of weight and there was only a slight increase in liver iron values. Also, the liver iron in some animals which received the corn grit diet with iron for long periods was greater than the total body iron of normal rats. On the basis of these data it was concluded that the normal intestinal block to iron absorption fails under certain conditions.

Experiments were designed to determine the dietary factors primarily involved in the failure of animals fed the corn grits and lard diet to control iron absorption. From these experiments it was concluded that the excessive absorption and deposition of iron in the livers of the animals on the diet are the result of the low phosphorus content of the diet. Further, it has been shown that it is possible to obtain excessive iron deposits in the livers of animals receiving a normal diet by adding large amounts of iron salts to the diet. These experiments indicate that non-absorption can be changed by altering either the phosphorus or the iron content of the diet. Analysis of the data obtained further indicates that the absolute amount of iron and/or phosphorus in the diet, as well as the iron/phosphorus ratio, influences the amount of iron absorbed.

RENAL EXCRETION OF CHOLESTEROL. Timothy Leary, Boston, Mass.

Abstract. In rabbits fed cholesterol the substance, in the form of crystalline esters, may appear in the kidney in tubular epithelial cells of the distal convoluted tubules and Henle's loops, first in the intermediate zone of the cortex and pyramids. Associated with this is the accumulation of cholesterophages in the intertubular connective tissue. Necrosis of these phagocytes may free crystalline esters, and splitting of the esters with disappearance of the fatty acids may lead to precipitation of solid cholesterol crystals. When feeding of cholesterol is continued for long periods, massive deposits of solid cholesterol crystals in the pyramids and the resulting fibrosis may cut off nephrons and give rise to nephrosclerosis. Observations under polarized light illustrate the presence of crystalline cholesterol in the lesions.

In human kidneys the excretion of crystalline ester cholesterol with damage to renal epithelial cells is manifested in glomerulonephritis, in lipoid nephrosis, and in lower nephron nephrosis. The appearance suggests that cholesterol is normally excreted in non-visible form, perhaps as ketosteroids. Polariscopic evidence of the excretion of visible forms of cholesterol is found almost exclusively in diseased kidneys.

CRYSTALLINE ESTER CHOLESTEROL AND RENAL ADULT CORTICAL TUMORS. Timothy Leary, Boston, Mass.

Abstract. Adult cortical renal tumors are found usually in persons, largely men, over 40 years of age, and in kidneys that tend to be nephrosclerotic. In such kidneys are sometimes found miniature papillary tumors, singly or in numbers, whose relation to renal cell tumors is close. In kidneys with multiple tumors fresh sections under polarized light disclose that the beginning and the progression of such a tumor is linked with the focal deposit of crystalline esters of cholesterol in cells of a proximal convoluted tubule causing the cells to swell. Then the affected portion of the tubule is sealed off from the rest of the tubule and undergoes cystic dilatation. The swollen cells proliferate. Blunt papillary stems are produced which begin to branch and then to produce a complex papillary new growth. In some

tumors the crystalline ester cholesterol is suddenly cast out of the tumor cells, and the tumor may stop active growth and subside to become a simple tubular adenoma, the cells of which contain no fat-staining material or crystalline ester cholesterol. In others, growth progresses, associated with evident focal accretions of cholesterol and with evidence of the removal of crystalline ester cholesterol piecemeal in macrophages. Steps in this progression and regression are illustrated.

Discussion

(Dr. Shields Warren, Boston, Mass.) It certainly would be worth while for all of us who run across these tumors to attempt in some small way to make studies of the type which Dr. Leary is carrying on. I think he has called our attention to a most interesting change, and the alteration in the cells that have been forced to depart from their normal functions to structural and perhaps organic change is of very real interest.

READ BY TITLE

ALLERGIC GRANULOMATOSIS. Jacob Churg and Lotte Strauss (by invitation), New York, N.Y.

Abstract. During the past 25 years a relation between allergic states and lesions of the type seen in periarteritis nodosa has been firmly established by experimental evidence and the study of human disease. Such lesions usually are limited to the blood vessels; there are, however, reports in the literature indicating that widely dispersed extravascular lesions may occur in the connective tissues. We have observed several cases of what might be considered typical periarteritis nodosa, accompanied by prominent lesions in the extravascular connective tissue, consisting of severe collagen alteration, exudation, and histiocytic proliferation. In another group of cases there were spent vascular lesions and still active extravascular changes. In one instance lesions of the same histologic type were limited to connective tissue while the vascular system showed practically no change. All 7 cases had a history of severe allergy, particularly asthma; furthermore, they ran a febrile course and showed blood eosinophilia.

A typical extravascular lesion had the appearance of a granulomatous nodule, often large enough to be seen with the naked eye. Histologically, it consisted of an eosinophilic core in which one could discern more or less severely altered collagen fibers mixed with varying numbers of disintegrating eosinophilic leukocytes. Where the collagen fibers could still be recognized they appeared markedly swollen and eosinophilic, and in polarized light they failed to show the usual double refractility. At times eosinophilic leukocytes were so numerous that one could not be sure of an underlying collagen change. The center of a nodule was surrounded by radially arranged epithelioid cells mingled with multinucleated giant cells of Langhans' or foreign body type, and beyond that, by a layer of exudate composed of eosinophilic leukocytes together with plasma cells and macrophages. One could observe various stages of evolution in the granulomas, from active exudation to more or less complete fibrosis. Calcification of the center occurred on occasion. In some cases there was associated eosinophilic pneumonia with giant cells, as seen in Loeffler's syndrome.

CODEINE POISONING: FATAL CASE FROM ACCIDENTAL OVERDOSE. V. H. Cornell, Washington, D.C.

Abstract. Death from codeine is so rare that only one case, that of an asthmatic patient, is found recorded. The present case is therefore reported primarily to record it for future reference and to indicate that this drug may be lethal in large doses.

A 31-year-old male accidentally drank 1 to 2 oz. of an improperly prepared codeine cough mixture, thus receiving between 13 and 26 gr. (0.9 to 1.75 gm.) of codeine sulfate. Concentrations found on toxicologic examinations were: brain, 0.5 mg. per cent; liver, 0.9 mg. per cent; urine, 12.5 mg. per cent; blood, 1.0 mg. per cent. The patient was an asthmatic and also under phenobarbital medication, the blood concentration of this drug being determined as 0.7 mg. per cent. An area of hemorrhage in the epicardium and auricular musculature in the region of the sino-auricular node may have been the final fatal lesion and probably was due to the anoxia induced by the medication. Death occurred in 12 to 15 hours.

ENCEPHALITIS IN DIPHTHERIA. Vera B. Dolgopol and (by invitation) Selig H. Katz, New York, N.Y.

Abstract. The report is based on the examination of the brains of 11 patients who died from 3 to 66 days after the onset of diphtheria. True encephalitis was found in 5 cases. The lesions consisted of (1) mononuclear infiltration of the venous walls in one or several locations (medulla, basal ganglia, frontal cortex, infundibulum), and (2) microglial nodules in the medulla (nucleus ambiguus, spinal vestibular nucleus). Severe cellular degeneration was rarely encountered, but scattered shrunken and hyperchromatic cells were frequent in the medulla. In another case no cellular infiltrates were found, but a few binucleated ganglion cells were seen in the medulla; this finding suggests that an inflammatory process had been present in the central nervous system at an earlier date. No demyelinization was observed in the brain tissue or in the nerve roots of these 6 cases. Myocarditis was present in 4 of the 6 cases, but apparently played no part in producing cerebral lesions, as none of them were of embolic character. Severe mental depression was observed in 1 patient, palatal paralysis was present in 2. Encephalitis in diphtheria could be the result of the action of the circulating toxin on the brain tissue and its blood vessels, or could be attributed to activation of some latent virus by the diphtherial toxin.

MALIGNANT HEMANGIOPERICYTOMA OF POPLITEAL SPACE. Robert Fienberg, Framingham, Mass.

Abstract. This case report concerns a 34-year-old white male with a mass of 8 months' duration behind the left knee. Biopsy revealed a malignant tumor, and a mid-thigh amputation was done. Examination of the surgical specimen showed a large lobulated mass involving the left popliteal space. The knee joint was clear. Microscopic examination revealed numerous thin-walled blood vessels surrounded massively by hyperchromatic cells with oval to round nuclei varying little in size and shape. No proliferation of endothelial cells was noted. The cells often grew in solid compact form. There was little necrosis. A reticulum stain showed a characteristic picture. There was marked invasion of the soft tissues of the popliteal space, including the striated muscle.

Since the hemangiopericytoma was first described by Stout and Murray in 1942, only a very few of the malignant variants have been described and published, so that very little is known concerning their characteristics. The slides of this specimen were examined by Drs. Fred W. Stewart and Arthur Purdy Stout, both of whom concurred in the diagnosis of malignant hemangiopericytoma.

SIMPLIFIED METHOD OF ALCOHOL ESTIMATION IN NON-DISTILLED VITAL FLUIDS. William E. B. Hall, St. Louis, Mo.

Abstract. With certain modifications, filtrates of protein-free blood and other materials are susceptible to colorimetric estimations of alcohol contents by the Duboscq colorimeter, preferably by the photo-electric colorimeter, without the in-

tervening steps of distillation, and particularly without the necessity of other substage titrations as presently used, as for instance, in the iodine titration method.

THE BASEMENT MEMBRANE OF THE FETAL PLACENTAL VILLUS AND ITS RELATION TO TOXIC AND PATHOLOGIC OBSTETRICAL STATES. William E. B. Hall, St. Louis, Mo.

Abstract. Except for a short presentation in an obscure medical publication, there appears to have been no identification or description of a basement membrane of the fetal placental villus. This structure has been identified as being histologically distinct, of a material related to, but not identical with, reticulum, and with staining qualities and characteristics similar to those of the vessels of the villi and the basement membrane of the renal glomerulus. This structure appears at approximately 1½ months of gestation and microscopically exhibits a pronounced affinity for calcium in certain placental degenerative states and with retention. Changes, particularly in the nature of thickening, are observed in toxic states, especially in association with intervillous thrombosis. The effect of thrombo-infarctions on the filtration function of the placental villus is reflected in the thickening and alterations of the basement membrane. This histologic and apparent functional change appears identical with that observed in the basement membrane in the various nephritides. The functional disturbance where the placental villus is so affected appears analogous to alterations in nephritic states and is expected to be accompanied by comparable toxic and hypertensive effects on the maternal and fetal organism, as well as distinct disturbances of the nutritional and excretory functions.

SQUAMOUS CELL METAPLASIA AND THE DIAGNOSIS OF LUNG CANCER. Samuel R. Haythorn, Pittsburgh, Pa.

Abstract. Squamous cell metaplasia of the bronchial epithelium has been found to be a relatively common lesion in the surgical and autopsy materials at the Allegheny General Hospital. It has been found in cases of bronchiectasis, abscessed lungs, organizing pneumonia, tuberculosis, and in non-cancerous portions of lungs showing squamous cell bronchogenic cancer. Squamous cell cancer has been the commonest type found in our series. A group of cases showing squamous cell metaplasia will be presented. They represent conditions in which the pathologist is frequently asked to make a differential diagnosis by the smear method. Desquamated epithelium from metaplastic areas may show prickles or cornification, and resemble squamous cancer cells closely. Where squamous cells are the only ones arousing suspicion in a smear for the diagnosis of bronchogenic cancer, a positive diagnosis should be made with reservations.

PLASMOCYTOMA OF THE THYROID. John B. Hazard and (by invitation) W. W. Schildecker, Cleveland, Ohio.

Abstract. Plasmocytoma of the thyroid is a rare disease, only one case having been reported during the last decade. The 2 cases to be described occurred in the material from 14,000 thyroid operations.

A man, 54 years of age, was admitted because of swelling of the neck of 5 years' duration. The thyroid was bilaterally markedly enlarged. A right lobectomy was performed. The capsule was intact and the lobe enlarged to 11 cm. in diameter. Histologically, there was a diffuse, dense infiltration by plasma cells, including giant forms. Radiation therapy was given and the remaining left lobe decreased markedly. Ten years later there was recurrence on the left due to an infiltrating plasma cell tumor. The lobe was removed piecemeal owing to marked capsular and muscle infiltration. Postoperatively, 3750 r. of radiation was given. The patient died of undetermined cause 6 years later, 21 years after the apparent onset of symptoms.

The second patient was a man, 71 years of age, with swelling of the face and neck of 6 weeks' duration. There was a marked enlargement of the left thyroid lobe with substernal extension. A left lobectomy was performed. The lobe was 12 cm. in diameter, weighed 240 gm., and had a thickened, shaggy capsule. The tissue was of soft consistence and yellowish white with irregular strands of lighter stroma. In most sections there was a diffuse infiltration of the thyroid by plasma cells, including rare giant forms. The thyroid parenchyma was markedly reduced, consisting of a few small oxyphilic follicles. Fibrous trabeculae and foci of lymphocytes were present in areas. Postoperatively, radiation therapy was given, totaling 1700 r. The patient was alive and well 5 years after operation.

Both patients were men, in the first instance without evidence of precedent thyroid disease, and in the second with areas in the thyroid suggestive of struma lymphomatosa (Hashimoto). Plasmocytoma of the thyroid, like other extramedullary types, may pursue a long course and show immediate response to radiation therapy, though in one of these cases there was recurrence after 10 years. In its first appearance the neoplasm revealed no penetration of the thyroid capsule, but with recurrence there was extension to adjoining muscle. In one instance the tumor was bilateral and in the other unilateral.

NECROSIS OF THE LIVER IN EXPERIMENTAL FATAL HEMOGLOBINURIC NEPHROSIS. Joseph J. Lalich (by invitation) and D. Murray Angevine, Madison, Wis.

Abstract. Variable degrees of hepatic necrosis have been observed in rabbits which died of hemoglobinuric nephrosis following starvation and dehydration and in addition received intravenous injections of hemoglobin. A total of 1.8 gm. per kg. of hemoglobin was given in 9 divided doses during the last 3 days of dehydration. Fifty-one rabbits have been treated in this manner. In this series, 17 of 18 rabbits died with severe hemoglobinuric nephrosis within 2 to 14 days following the first injection of hemoglobin. Of the 34 surviving animals, all had pigment casts in the renal tubules from 8 to 14 days after the initial hemoglobin injection. Hepatic necrosis was observed in 12 of 17 rabbits which died, but was not observed in animals which survived the injections.

Hemoglobinuric nephrosis was considered severe when 100 or more pigment casts were present in 10 low-power fields. The hepatic necrosis varied considerably in extent. In 4 instances the areas of necrosis were confluent and 25 per cent or more of the liver was involved. From 8 to 20 areas of necrosis per $cm.^2$ of section were seen in 4 livers and 1 to 3 areas per $cm.^2$ in 4 others. The liver necrosis may occur focally; however, in most instances it is central. Additional alterations frequently observed were congestion of the central vein and liver sinusoids, dilatation of lymphatics, and vacuolization of cytoplasm of liver cells. Increased pigment deposition was observed also, either in the liver cells or the Kupffer cells, in approximately one-half the instances in which hepatic necrosis occurred.

It is not apparent from these studies what factors are responsible for the hepatic necrosis. The observation that 5 rabbits died of hemoglobinuric nephrosis without evidence of liver necrosis suggests that the renal changes occur first. This study corroborates previous observations that dehydration and starvation are precipitating factors for the development of hemoglobinuric nephrosis, and further suggests that the same factors are probably concerned in the production of hepatic necrosis.

COMPLEMENT FIXATION IN ANIMAL NEOPLASIA. III. COMPARISON OF THE KIDD TECHNIC WITH A MORE SENSITIVE METHOD FOR TESTING THE SERA FROM RABBITS BEARING THE BROWN-PEARCE CARCINOMA. Stuart W. Lippincott and (by invitation) Lester D. Ellerbrook, Helen Thornton, and Ellery C. Stowell, Seattle, Wash.

Abstract. Kidd has reported on a complement-fixing antibody in the sera of certain rabbits of various breeds inoculated with the Brown-Pearce carcinoma. His

report has been substantiated by our studies and in addition a more sensitive and quantitative method for measuring the antibody has been devised. Definite improvements in standardizing the technic have resulted from using the amount of complement giving 50 per cent hemolysis as the unit instead of the 100 per cent unit. Optimum conditions of the test have been investigated with respect to methods of preparation and purification of the antigen, use of the proper ratio of red cells and hemolysin, and time and temperature of fixation.

Sera were obtained before inoculation of the neoplasm and at daily or weekly intervals thereafter up to 100 days. Other control sera were obtained from non-tumor-bearing rabbits from those inoculated with autoclaved and frozen Brown-Pearce tumor, with normal rabbit tissue, and with such substances as human albumin and turpentine. Some of the antigens prepared from the neoplastic tissue were the following: saline extracts, buffered saline extracts, and sediment and supernate from the saline extract centrifuged in a Sharples supercentrifuge for 60 or 90 minutes at 45,000 r.p.m. (50,000 g. at the periphery).

A number of postinoculation sera giving negative reactions by Kidd's technic gave perceptible reactions when his conditions of fixation were changed from 2 hours at room temperature to 18 hours at 5° C. Some sera which were negative by the latter procedure gave positive reactions by our standardized technic, *i.e.*, fixation for 18 hours at 5° C. in the presence of graduated known amounts of complement. The extent of the reaction was measured by the difference between the amounts of complement required to give 50 per cent hemolysis in the test and in the controls. The pre-inoculation sera showed no definite fixation of complement whereas most sera showed a definite positive reaction after the neoplasm had grown for 2 to 3 weeks.

THE INCIDENCE OF POST-TRANSFUSION SERUM HEPATITIS. John J. McGraw, Jr. (by invitation), Max M. Strumia, and Mary Elizabeth Burns (by invitation), Bryn Mawr, Pa.

Abstract. Serum hepatitis (homologous serum jaundice) is transmitted by injection of human blood and certain fractions of blood. The incidence of hepatitis following transfusions of blood and plasma is a matter of considerable importance in transfusion therapy. An attempt has been made to determine the incidence of serum hepatitis at the Bryn Mawr Hospital during a period of approximately 4 years starting in December, 1943. Complete records of all blood donations, plasma pools, and transfusion were available. The pools contained plasma of 8 to 12 donors. Letters and questionnaires were sent to 1,523 transfused patients. Identical letters and questionnaires were sent to 1,300 non-transfused patients who were admitted to the hospital in the same period. The latter were used as controls. The questionnaire simply asked if the patient developed jaundice in a period dating 6 months from the time of hospitalization.

Nine hundred and thirty-six transfused patients and 1,000 controls responded. The answers were analyzed, hospital records reviewed, and the attending physicians were contacted. Quite a number of the affirmative answers in both series were discarded because the jaundice referred to by the patient was present at the time of admission to the hospital. These were eliminated and all cases of jaundice which had their onset in the 6 month study period were classified as "possible serum hepatitis" even though some patients were known to have reason for bile duct obstruction. Further study permitted elimination of some cases as almost certainly due to common duct stones, carcinoma of the pancreas, portal cirrhosis, etc. The remaining cases were classified as "probable serum hepatitis."

In the 936 transfused patients there were 20 cases (2.12 per cent) classified as "possible hepatitis." Twelve cases (1.29 per cent) were considered as probably

due to the transfusions. In the 1,000 controls there were only 2 cases (0.2 per cent) of "probable hepatitis" in the 6 month study period.

The cases were further analyzed to determine the incidence of serum hepatitis following whole blood as opposed to pooled plasma. As might be expected, the rate was higher in those patients who received pooled plasma. In the group of 382 patients who received nothing but whole blood, there was only one case of "probable hepatitis" (0.26 per cent). In the group of 528 patients who received pooled plasma, with or without blood, there were 11 cases (2.08 per cent) of probable hepatitis.

Our findings of a 1.29 per cent incidence of probable hepatitis following blood or plasma and a 2.08 per cent incidence following plasma with or without whole blood are based upon the number of cases of hepatitis per transfused patient. Since many patients received multiple units of blood plasma, the incidence of hepatitis per transfusion is much lower. The 936 patients who responded to the survey received a total of 3,723 transfusions. The incidence per transfusion of blood or plasma was approximately 1 in 300. In the group who received Bryn Mawr plasma there were 2,350 such transfusions and the incidence of hepatitis was 1 for each 235 units administered.

Although the findings revealed in this survey indicate that serum hepatitis is a rather rare complication of plasma transfusion when the pools are relatively small, it is planned to sterilize our plasma with either ultraviolet light or nitrogen mustard and, after a period of a few years, repeat the survey on a new group of patients.

TISSUE CHANGES CLOSELY RESEMBLING AMYLOIDOSIS IN SCORBUTIC GUINEA-PIGS. Conrad L. Pirani (by invitation) and Chauncey G. Bly (by invitation), Chicago, Ill.

Abstract. In the course of a pathologic study of chronic scurvy, deposits of amyloid-like material were observed in various organs.

Thirty young guinea-pigs were fed scorbutogenic diets for varying lengths of time; 12 received the Sherman-LaMar diet as modified by Rinehart, and 18 the Crampton no. 5 diet. The control animals received 2 mg. of ascorbic acid daily, the acute scurvy animals none, and those of the chronic scurvy group 0.2 mg. Amyloid-like material was found only in the chronic scurvy guinea-pigs and in only those animals which were sacrificed after 8 weeks or longer from the beginning of the experiments. Seven of 8 animals in this last group showed distinct deposits of amyloid-like material. In these cases the spleen was severely affected, the liver moderately, and the adrenal cortex only minimally. No other organ appeared to be involved.

Microscopically, this material appeared in the spleen in the form of a thick band in the peripheral portions of the malpighian corpuscles. In the liver it appeared in moderate amounts between the hepatic cords and the wall of the sinusoids with resulting compression and atrophy of the liver cells. In the adrenal cortex small amounts were noted in close apposition with and at times completely surrounding the walls of capillaries. Iodine and sulfuric acid tests on the gross specimens, and crystal violet and congo red stains on the sections, were all positive. The tinctorial response of this amyloid-like substance with congo red, however, was not as brilliant as is usually seen in pathologic specimens from man. In sections stained with congo red and examined with polarized light, birefringence was observed in focal areas where the amyloid-like deposits were deeply stained. The organs involved, the typical distribution of this substance in the immediate neighborhood of capillaries and of reticulo-endothelial elements, and the results of the tests with special stains are those usually observed both in human amyloidosis and experi-

mental amyloidosis in animals. No definite conclusions, however, can be reached at this time as to the identity of the substance observed in these experiments with human amyloid.

THE EXFOLIATIVE CYTOLOGIC DIAGNOSIS OF CENTRAL NERVOUS SYSTEM LESIONS. William R. Platt, Camden, N.J.

Abstract. Using the same fundamental concept described by its earliest proponents, *i.e.*, that many malignant tumors will ultimately desquamate tumor cells onto a presenting free surface, the author examined the aspirated fluids obtained from the lateral ventricles, cisterna magna, spinal canal, and cystic brain masses from patients with proved and with clinically diagnosed central nervous system entities. The microscopic patterns of these are to be presented and discussed.

EXPERIENCE WITH UNSUCCESSFUL ATTEMPTS TO USE THE ANTERIOR CHAMBERS OF EYES OF HETEROLOGOUS SPECIES TO GROW MALIGNANT NEOPLASMS AS A SOURCE OF ANTIGEN. Walter Ricker (by invitation), Seattle, Wash.

Abstract. The possibility of growing malignant neoplasms in the anterior chamber of guinea-pig and rabbit eyes has been investigated as a possible source of neoplastic tissue to be used for the preparation of antigens in a study of complement fixation in animal neoplasia. In an experience of 2 years the method has been found unsuitable for this purpose. Seven types of malignant neoplasms were used for transplantations. The eyes of the alien species into which transplants were made were examined for gross evidence of growth throughout the experiments, and at death or sacrifice each eye to which transplantation had been done was removed, fixed in formalin, embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin. These sections were examined microscopically for residual or growing tumor. The neoplasms transplanted were: (1) Two C_3H mouse mammary carcinomas transplanted to 19 guinea-pig eyes and 21 rabbit eyes and observed for a period of 80 days. (2) Five Brown-Pearce rabbit carcinomas transplanted to 42 guinea-pig eyes, 2 of which were retransplanted to 4 other guinea-pig eyes and observed for a period of 69 days. (3) Three adenocarcinomas of human breast origin transplanted to 5 rabbit eyes and 22 guinea-pig eyes, with 1 subsequent retransplant to 1 guinea-pig eye and observed for a period of 212 days. (4) One spontaneous squamous cell carcinoma of mouse skin transplanted to 3 guinea-pig eyes and observed for a period of 192 days. (5) One spontaneous mouse hepatoma transplanted to 4 guinea-pig eyes and observed for a period of 189 days. (6) One spontaneous adenocarcinoma of cat breast transplanted to 3 guinea-pig eyes and observed for a period of 102 days. (7) Two transplanted "L" strain fibrosarcomas to 49 guinea-pig eyes and observed for a period of 103 days.

Six eyes to which the C_3H mammary carcinoma was transplanted and 5 eyes to which the Brown-Pearce carcinoma was transplanted showed proliferative changes interpreted as consistent with gross growth. Twenty-eight of the 49 guinea-pig eyes to which the "L" strain fibrosarcoma was transplanted showed changes consistent with gross growth. Of this total of 39, 4 of the eyes to which the "L" strain fibrosarcoma had been transplanted showed, on the 8th day following transplantation and by microscopic examination, neoplastic growth consistent with that observed in sample sections of the original neoplasm. All other microscopic findings were exudative or proliferative, predominantly the latter.

Of 173 transplants of 7 types of malignant neoplasms, 147 to the anterior chambers of guinea-pigs' eyes and 26 to the anterior chambers of rabbits' eyes, 39 showed changes at some interval considered consistent with gross growth of the transplant. Microscopic examination of all of the eyes corroborated the gross observation in only 4 instances in the "L" strain fibrosarcoma.

METHYLCHOLANTHRENE-INDUCED TUMORS IN GUINEA-PIGS WITH TRANSIENT PERIODS OF VITAMIN C DEFICIENCY. William O. Russell and (by invitation) Leonard R. Ortega, Houston, Texas.

Abstract. Because of the infrequent use of the guinea-pig in cancer research and the fact that these animals are generally believed to be refractory to carcinogenic agents, these experiments were undertaken to further evaluate this animal with regard to experimental carcinogenesis. Ninety-eight young, but mature, male and female guinea-pigs were injected subcutaneously in the right axilla with 30 mg. of methylcholanthrene dissolved in sesame oil. The animals were divided into 3 groups for the experiment. One group of 28 animals was a normal control and received a full diet, a second group of 31 animals received a full but restricted diet to control inanition, and a third group of 39 animals received periodically a scorbutogenic diet.

Seventy-three guinea-pigs lived past the appearance of the first tumor (170 days). Thirty-nine of these developed tumors at the site of the methylcholanthrene injection. Seventeen of the tumors were fibrosarcomas, 10 of them were osteogenic sarcomas, and 12 were liposarcomas. Although many of the tumors showed extreme anaplasia and evidence of rapid growth, metastases were not observed.

Fifteen tumors were produced in the scorbutic group, 12 in the inanition control group, and 12 in the normal controls. The average induction period for each group was as follows: 242 days in the scorbutic animals, 295 days in the inanition control animals, and 393 days in the normal controls. There was no observable difference in the histologic types seen in the 3 groups. The length of the induction period was remarkably shorter in the scorbutic group. These results are not statistically significant in regard to a possible effect of vitamin C deficiency on influencing the induction of the tumors. The shortened induction period in the scorbutic and inanition group is statistically significant.

OLD ENDOCARDITIS IN THE HEARTS OF PIGS RESEMBLING HUMAN ENDOCARDITIS. Otto Saphir and (by invitation) Martin Lowenthal, Chicago, Ill.

Abstract. In a study of 950 hearts of pigs, old endocarditis was encountered in 10 per cent. These resembled grossly and microscopically old endocarditides in humans, often interpreted as the result of rheumatic fever. Since *Erysipelothrix rhusiopathiae, Salmonella suipestifer,* swine plague, and hog cholera infections with resulting endocarditis occur in a relatively high percentage of pigs, it is very likely that the old endocarditis encountered here is the result of a healed endocarditis caused by one of the above organisms, the most frequent being *Ery. rhusiopathiae.* Since this healed endocarditis is similar in many respects to human rheumatic endocarditis, the question is raised whether some instances interpreted as rheumatic endocarditis are not the result of a healed bacterial endocarditis of unknown origin.

AN EVALUATION OF THE ADAMSTONE-TAYLOR FROZEN SECTION TECHNIC FOR USE IN ROUTINE SURGICAL PATHOLOGY. Edgar B. Taft (by invitation), Kansas City, Kansas.

Abstract. A method for the rapid preparation of permanent, thin, frozen sections was described recently by Adamstone and Taylor (*Stain Technol.,* 1948, 23, 109–116). With this technic, sections of fresh, unfixed tissue are made and kept frozen until they are attached to microscopic slides, ready for processing. There are several differences from the ordinary frozen section technic: The microtome knife is cooled with blocks of dry ice; the sections are transferred from the knife blade to slides by means of a hollow, shovel-shaped scoop filled with

shaved dry ice; and the slides are immersed in fixative just as the tissue sections began to melt.

An old Leitz sledge-type microtome and a clinical frozen section microtome head were combined and adapted to the technic as described by its originators. Fresh tissue was obtained from the operating room or from the Pathology Department, usually within 4 hours of the time at which it was removed from the patient. Sections were made from blocks of the usual size, sometimes simultaneously from two organs such as an ovary and oviduct, and fixed in a variety of standard fixatives. Sections so obtained could be fixed, stained, and mounted permanently within 15 minutes from the time the block of tissue was frozen and doubtless could have been treated even more rapidly if it had been desirable.

There are a number of disadvantages inherent in this method. The most obvious is that sections cannot be made if the room temperature exceeds 65° to 68° F. In the winter this difficulty is easily overcome; in warm weather, even with air-conditioning or a cool basement room, such a low temperature might not be achieved readily. Considerable manual dexterity is necessary for this technic, and two persons must participate in making the sections. Bone and calcified tissue, of course, cannot be examined by this method. It also has been found that tissues with a large amount of fat, such as a lipoma or very fatty breast, do not section satisfactorily. Firm tissues with moderate to large amounts of dense collagen such as cervix uteri or leiomyoma from postmenopausal patients likewise have been sectioned with difficulty. Scirrhous carcinoma of the breast, on the other hand, has yielded excellent sections.

The advantages of this method are numerous: Permanent, thin (4 to 6 μ), flat sections are obtained; sections for possible fat stains or histochemical determinations can be cut from the same block as the routine sections and at the same time; and sections of such tissues as lymph node, intestinal tract and spleen can be made with ease. However, it must be admitted that the most satisfactory sections are obtained from such tissues as liver, brain and kidney, so that, except in a few instances, this method is perhaps best adapted for use in experimental laboratories.

HISTOLOGIC OBSERVATIONS ON THE SUPRADIAPHRAGMATIC PORTION OF THE VAGUS NERVES. C. G. Tedeschi and E. A. Gaston (by invitation), Boston, Mass., and Framingham, Mass.

Abstract. The purpose of this communication is to present the microscopic findings in specimens of vagus nerve removed at operation from patients with duodenal ulcer, and in control series from post-mortem material. Reports of nerve injuries frequently include evidence of damage to peripheral nerves without loss of anatomic continuity. A spindle-shaped neuroma, a perineural thickening, a dense intraneural fibrosis or scarring is often found under such circumstances and to these lesions is traced the lasting loss of excitability. This gamut of changes and occasionally neurolysis and inflammatory cell infiltration were shown, with unexpected frequency, by the vagotomy specimens in our series. These changes were focal in distribution, the whole resulting in an irregular alternation often in the same microscopic field, of apparently intact fascicles and of fascicles displaying one, or a combination, of the above outlined changes.

A number of investigators have shown the susceptibility of the peripheral nerves to minimal trauma, including pressure and transient stretching. The nature of the changes encountered in our series speaks against the possibility that they resulted from stretching or pressure applied to the neural structures at the time of operation. Neuronal changes similar to those seen in the segments of vagus nerves removed at operation from patients with duodenal ulcer were occasionally seen in corresponding segments of vagus nerves obtained at autopsy in the absence of any

evidence of gastro-intestinal disease. The number of specimens studied up to the present time, 19 surgical and 32 post-mortem, is too small to be statistically significant. However, a comparative analysis of the findings in the surgical and in the control series revealed changes to be much more frequent in the former. Assuming that the normal function of a nerve is dependent upon the proper transmission of impulses, the possibility must be entertained that changes in the vagal nerves, such as those seen in some of our cases, may play some part in the development of disorders of structure or function occurring in the alimentary tract.

ACID-FAST INTRANUCLEAR INCLUSION BODIES IN LEAD POISONING. M. Wachstein, Brooklyn, N.Y.

Abstract. Intranuclear inclusion bodies occur after the application of various metallic compounds. Their unexpected presence in tissue sections may occasionally lead to the correct interpretation of an unexplained death. This is shown by the following observation.

A 21-month-old boy had shown increasing irritability for 4 weeks. Forty-six hours before his death he was admitted because of prolonged vomiting. During his stay in the hospital he developed drowsiness, signs of meningeal irritation, and died in coma. Significant laboratory findings consisted of an increased amount of protein (258 mg. per cent) in the spinal fluid with a cell count of 25, of which 90 per cent were polymorphonuclear leukocytes and 10 per cent lymphocytes, and evidence of considerable anemia with a few basophilic stippled red cells.

Gross autopsy findings were essentially negative. In sections of the kidneys and to a lesser degree in those of the liver, innumerable acidophilic intranuclear inclusion bodies were found. Chemical tests performed on the basis of these findings in a composite sample of kidney and liver showed 1.5 mg. of lead in 100 gm. of wet tissue (upper limit 0.05 mg. in control material).

Simular inclusion bodies, first described by Blackman, were seen in rats given a lead-containing diet. All of these inclusion bodies were acid-fast. Among various other inclusion bodies examined, only those following bismuth application proved to be likewise acid-alcohol resistant to decolorization after staining with carbolfuchsin. The renal inclusion bodies following bismuth application are, however, easily distinguished on the basis of their different morphologic characteristics.

INFARCTION OF THE MUSCLE BUNDLES OF THE ATRIA AND VENTRICLES OF THE HEART. William B. Wartman and (by invitation) John Souders, Chicago, Ill.

Abstract. Fifty hearts with infarcts were examined by cutting each like a loaf of bread from the apex to the base by means of multiple parallel transverse cuts. The distribution of the infarcts was compared with the known distribution of the cardiac muscle bundles. In every instance the pattern of the infarct coincided with the pattern of either a single muscle bundle or of a group of bundles. The entire muscle bundle was never involved but only a segment of it. Infarcts occurred in the superficial muscle bundles, either singly or in combination, in half the cases. In nearly one-fourth of all cases both superficial bundles were affected. The superficial sinospiral muscle contained infarcts twice as commonly as the superficial bulbospiral muscle. In 10 cases the superficial bundles were involved in conjunction with deep muscles. Thus the superficial muscle bundles were the seat of infarcts, either singly or in combination, in 71 per cent of cases. Deep muscle bundles were affected 22 times (48 per cent). In 6 cases both deep muscles were affected and in 10 cases deep muscles were affected in combination with the superficial muscles.

Infarcts were found in the atria in 14 hearts (29 per cent). Most of these were in the right auricle and were recent, suggesting that they may have contributed to the death of the patients.

It was found in general that when the right coronary artery was occluded an infarct developed in a deep muscle; when the descending branch of the left coro-

nary artery was occluded an infarct developed in one or both of the superficial muscles. When the circumflex branch of the left coronary artery was occluded the infarct developed in either deep or superficial muscles. When multiple occlusions occurred in different coronary arteries there was great likelihood that more than one muscle bundle would be the seat of infarction.

It was possible to classify infarcts into three types depending upon the thickness of the ventricular wall which was involved. Type 1, or full thickness infarcts, included those in which the entire thickness of the ventricular wall was necrotic. Type 2, or massive infarcts, involved a large mass of muscle, but not the full thickness of the ventricular wall. Type 3, or laminar infarcts, involved only a portion of a single muscle. Full thickness and massive infarcts occurred with nearly equal frequency (approximately 36 per cent for each type). The remainder (approximately 25 per cent) were laminar. Rupture occurred only in hearts with full thickness infarcts. However, the fact that not all hearts with full thickness infarcts ruptured indicates that other factors, such as blood pressure, and the degree of liquefaction of the infarct, were also important. Ninety per cent of cardiac aneurysms occurred either with full thickness or massive infarcts and only 10 per cent with laminar infarcts.

When the infarct was of full thickness type, two or more bundles were involved in most cases (13 of 17). Massive infarcts involved two or more bundles in 13 instances and a single muscle in 7. The situation was reversed in the case of laminar infarcts. There were 8 in which a single muscle was affected and only 3 in which 2 muscle bundles were involved. Fifty per cent of the hearts had an old infarct and of these all but 4 also had a new infarct. A striking feature was the frequency with which the new infarct occurred in the same area as the old one.

BILATERAL MAMMARY ARTERITIS. Theodore R. Waugh, Montreal, Canada.

Abstract. A case is reported of a 63-year-old woman who developed a lump in the right breast due to an inflammatory condition of a branch of the mammary artery. This was followed 2 months later by a similar lesion of the left breast. The histopathologic changes in the vessels and clinical course of the disease are similar to those met with in temporal arteritis.

COLOR REACTIONS WITH SERA OF PATIENTS WITH MALIGNANT NEOPLASMS. Emil Weiss, Chicago, Ill.

Abstract. A number of aniline dyes in dilutions of 1:10,000 do not show any color changes on addition of sera from patients with cancer. Sera of normal individuals and of those afflicted with various diseases or benign tumors change to green the original blue color of the same dyes. The glassware must be clean and free of any acid or dye. The sera must be fresh and thoroughly centrifuged. Turbid, hemolyzed, or icteric sera cannot be used. The dyes are kept in 0.1 per cent stock solutions of rubbing alcohol (70 per cent isopropyl alcohol). The stock solution is diluted with distilled water 1:10 before use. To 0.3 cc. of the diluted dye 0.5 cc. of serum is added, the tube is shaken for a few seconds, and the color changes are noted. A positive control containing a known malignant serum and a negative control containing a known normal serum are handled in the same manner. A dye control, correspondingly diluted with water, also is used. The tubes are placed overnight or for 12 hours in the refrigerator and then the final reading is made. Eventual color changes become more distinct after standing several hours. Over 300 sera were examined with the above technic. Positive (blue) reactions were obtained in 89.1 per cent of sera from patients with malignant neoplasms. Azur II, toluidine blue, trypan blue, and Victoria blue were found to be equally useful. This method applies to all types of cancer. Hodgkin's disease and various forms of leukemia give consistently positive results.

XU